Metabolism of lactose-[\textsuperscript{13}C]ureide and lactose-[\textsuperscript{15}N,\textsuperscript{15}N]ureide in normal adults consuming a diet marginally adequate in protein

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**ABSTRACT**

Oral lactose-ureide is resistant to human digestive enzymes, but is fermented by the colonic microflora. Nine normal adults consuming a diet which provided 36 g of protein/day were given oral doses of lactose-[\textsuperscript{13}C]ureide and lactose-[\textsuperscript{15}N,\textsuperscript{15}N]ureide. The appearance on breath of \textsuperscript{13}CO\textsubscript{2} derived from lactose-[\textsuperscript{13}C]ureide was followed for 48 h. The fate of \textsuperscript{15}N derived from lactose-[\textsuperscript{15}N,\textsuperscript{15}N]ureide was determined by measuring the recovery of \textsuperscript{15}N in stools and urine in various forms. About 80\% of the label given as lactose-[\textsuperscript{13}C]ureide was recovered on the breath, and about 80\% of label given as lactose-[\textsuperscript{15}N,\textsuperscript{15}N]ureide was not recovered in stool, indicating that 80\% of the dose was completely fermented. At least 5\% of the labelled urea was absorbed and excreted as the intact molecule. Of the \textsuperscript{15}N derived from lactose-[\textsuperscript{15}N,\textsuperscript{15}N]ureide and available for further metabolic interaction, 67\% was retained and 33\% was excreted in urine. The time taken for [\textsuperscript{15}N,\textsuperscript{14}N]urea to appear in urine was similar for all subjects, but the appearance of either \textsuperscript{13}CO\textsubscript{2} on the breath or [\textsuperscript{15}N,\textsuperscript{14}N]urea in urine varied. It is concluded that the hydrolysis of the sugar–urea bond may reflect oro–caecal transit time, but that other factors related to colonic bacterial metabolism determine the duration and extent of hydrolysis of urea by urease enzymes. Lactose-ureide can be used to probe the metabolic activity of the colonic microflora in normal individuals.

**INTRODUCTION**

The colon is host to a rich and complex microflora which engage in extensive metabolic exchange. It is increasingly appreciated that microflora play a role in intermediary metabolism [1]. For example, complex carbohydrates which escape digestion may be fermented by the microflora, with the production of metabolic intermediates such as short-chain fatty acids, which may help maintain the health of the colonic mucosa [2]. However, the extent of this interaction with the host has only been explored to a limited extent, because of difficulties in probing metabolic exchange directly. We have been interested in the extent to which the colonic flora contribute by helping to salvage urea-nitrogen and thereby achieving economies in nitrogen utilization [3]. Increased colonic salvage of urea-nitrogen appears central to the nitrogen economy of the body when the metabolic demands for protein are increased, such as during growth, or when the dietary provision is reduced or limiting [4]. In the studies in which colonic salvage of urea-nitrogen has been assessed, indirect approaches have been used to determine the magnitude of the colonic exchange by difference [3,4]. The only opportunity to access the colonic lumen directly has required either that advantage be taken of the relative ease of access in people with a colostomy [5], or by accessing the lumen during colonoscopy [6]. Although these approaches provide useful information, neither can be used to assess normal function. People with a colostomy clearly have had some form of colonic

Key words: amino acids, colon, nitrogen, protein, stable isotopes, urea.

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Scheme 1  Bacterial metabolism of sugar-ureide in the colon

The sugar–urea bond of lactose-ureide is resistant to digestion, but is fermented by bacterial enzymes. Allantoate amidinohydrolase splits the bond between the sugar and urea to release urea, which is hydrolysed by urease. When a stable isotopic label (¹⁴C or ¹⁵N) is placed on either the carbon or nitrogen of urea, the potential fate can be followed.

pathology, and in order to carry out colonoscopic investigation fairly harsh preparation of the bowel is required.

Ideally we would want to be able to present a suitable metabolic probe to the colonic lumen in normal individuals, and to be able to follow the fate of that material. One possible approach of interest would be to adopt a method that has been used to assess gastrointestinal transit time [7,8]. The bond between urea and the sugar moiety in lactose-ureide is completely resistant to the body’s digestive processes, although the action of lactase may release galactose and glucose-ureide [9]. Therefore, following an oral dose of lactose-ureide, the urea is locked as a sugar-ureide molecule which traverses the small intestine to be presented to the colon. Despite its resistance to human digestive enzymes, the sugar-ureide is fermented by bacterial enzymes (Scheme 1). It is thought that, in the first instance, bacterial allantoate amidinohydrolase (EC 3.5.3.4) breaks the sugar–urea bond to release the sugar and urea. The released urea is then available to be acted on by bacterial urease (EC 3.5.1.5), with the generation of ammonia and carbon dioxide [7]. If atoms within the urea moiety are suitably labelled with stable isotopes, it should be possible to determine the further fate of these elements within the body [8]. We have used this approach to deliver a defined dose of labelled urea to the colon and, by collecting samples of breath, urine and stool, have determined the relative disposal of label to each of these fates; furthermore, we have determined by difference the retention of urea-nitrogen in the body. Lactose-[¹⁵C]ureide has been administered on the assumption that the recovery of ¹⁵CO₂ on breath indicates the extent to which urea has been hydrolysed following release from lactose-ureide. Lactose-[¹⁵N,¹⁴C]ureide has been administered to trace the metabolic fate of urea-nitrogen (Scheme 1) [8].

The nitrogen economy of normal adults consuming a diet low in protein is enhanced by the metabolic activity of the colonic microflora that hydrolyse urea, leading to an increase in the salvage of urea-nitrogen. Thus, in order to increase the likelihood that a significant amount of label was retained in the body, the studies were carried out with subjects consuming a diet low in protein [10].

METHODS

Subjects
The studies were carried out in nine normal adults (six females and three males; age 19–26 years), who were members of staff at our institute. All were in good health at the time of the study, none had been on antibiotics for the 2 months prior to the study, and none consumed recognized probiotics. The study was approved by the Southampton Hospitals and South West Hampshire Health Authority Ethical Subcommittee. Each subject agreed to participate after the nature of the investigation had been explained.

The study period lasted 7 days, during which the subjects consumed a diet that had been prepared for them. The diet was marginally adequate in protein (approx. 36 g/day), and provided 188 kJ day⁻¹ kg⁻¹ (45 kcal day⁻¹ kg⁻¹). The food was ingested as three main meals, and each subject kept a record of all food consumed, including soft drinks and boiled sweets, which they were allowed to consume ad libitum. The metabolic investigations were carried out on days 4 and 5, during which time the subjects were admitted to the Clinical Nutrition and Metabolism Unit at Southampton General Hospital.

Experimental protocol
On day 4 of the study, meals of similar energy and protein content were consumed every 3 h from 06.00 hours for 15 h. The study was designed to follow the recovery on breath of ¹⁵CO₂ derived from the ingestion of a single oral dose of lactose-[¹⁴C]ureide, and to determine the recovery in urine and stool of ¹⁵N derived from the ingestion of prime and intermittent oral doses of lactose-[¹⁴N,¹⁵N]ureide. At 06.00 hours on day 4 of the study, samples of breath and urine were collected to determine baseline abundance. A single dose of lactose-[¹³C]ureide (500 mg, dissolved in water) was then ingested by each subject before eating any food. Excretion of CO₂ on breath was measured with an indirect calorimeter using a ventilated hood system (Deltatrac; Datex Instrumentation Corp., Helsinki, Finland) for 10 min every 1 h from 06.00 hours until 23.00 hours. At the same times, samples of end tidal breath were collected.
to a breath bag (Quintron, Milwaukee, WI, U.S.A.), and were also collected at 24 h, 30 h, 36 h and 48 h after the administration of the dose of lactose-[15N]urea. Aliquots of breath (10 ml) were transferred in triplicate into evacuated tubes (Europa Scientific Ltd., Crewe, U.K.) for the measurement of enrichment. At 06.00 hours, a priming dose of lactose-[13C15N]ureide (3.21 mg/kg body weight) was taken orally, followed by intermittent oral doses of 0.64 mg/kg every 3 h for 18 h. Urine was collected into acidified containers every 3 h for 18 h and stored frozen until analysed. A sample of stool was collected from each subject on day 3, and all stools passed during days 4–7 were collected into polythene bags and immediately frozen at −20 °C. Individual samples were weighed and homogenized with a known volume of deionized water for 1–2 min, and an aliquot of known mass was stored at −20 °C until further analysis.

**Synthesis of lactose-ureide**

Lactose-[15N,15N]ureide and lactose-[13C]ureide were synthesized from [15N,15N]urea and [13C]urea (99% atoms 15N, 99% atoms 13C; Cambridge Isotope Laboratories, Cambridge, MA, U.S.A.) respectively, by the method of Hofmann [11], with a yield of about 50%. Samples were recrystallized, and purity (assessed by gas chromatography-MS, electrospray-MS and reaction with urease enzyme) was estimated to be greater than 99%.

**Analyses**

**Diet**

The subjects were provided with, and consumed, a diet designed to contain approx. 36–38 g of protein/day. The diet was made up of normal foods, and the composition was calculated from food composition tables with the use of a computerized database [12] (Comp-Eat; Nutrition Services, London, U.K.). The consumption of energy and protein for each subject during the study period is shown in Table 1.

**Breath**

The isotopic enrichment of CO2 in breath was determined by isotope ratio MS using an automated breath system (20/20 with GLS interface, continuous flow; Europa Scientific).

**Urine**

The nitrogen content of urine was determined by Kjeldahl analysis. The concentration of urea and ammonia was measured using the Berthelot method. Urea nitrogen was isolated for MS using short-column ion-exchange chromatography [13]. A volume of urine estimated to contain 2 mg of urea was brought to pH 2 with HCl, using Cresol Green as an indicator. This was loaded on to a column of resin (1 cm × 5 cm; Dowex 50-WX8-200 mesh; H+ form), and washed on with 2 ml of deionized water. A disodium citrate buffer, pH 3.1, was used for elution. The eluate containing urea (between 10 and 35 ml) was collected and brought to pH 12 with NaOH (40 g/100 g) and the volume was reduced by boiling. Nitrogen gas was liberated from urea by reaction with alkaline hypobromite in vacuo, in a monomolecular reaction in which the two nitrogen atoms from a single molecule of urea give rise to a single molecule of nitrogen gas [14]. Hence the relative proportions of [15N,15N]urea, [15N,14N]urea and [14N,14N]urea in solution result in the same proportions of [15N,15N], [15N,14N] and [14N,14N] in the gas generated. The nitrogen generated was dried, and possible contaminating gases were removed in a liquid nitrogen trap, before being entered into the mass spectrometer. The proportions of nitrogen gas of different mass were determined in a triple collector isotope-ratio mass spectrometer (SIRA 10; VG Isogas, Winsford, Cheshire, U.K.).

**Stool**

Portions of faecal homogenate (30–40 g) were freeze-dried to constant weight (Genevac, Ipswich, U.K.).

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**Table 1 Age, height, weight, body mass index (BMI) and basal metabolic rate (BMR) of individuals who participated in the study**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>BMR (kJ/day)</th>
<th>Energy (kJ/day)</th>
<th>Protein (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>23</td>
<td>1.91</td>
<td>75.0</td>
<td>20.6</td>
<td>7672</td>
<td>12650 ± 350</td>
<td>37.5 ± 2.0</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>23</td>
<td>1.70</td>
<td>62.5</td>
<td>21.6</td>
<td>5916</td>
<td>10700 ± 120</td>
<td>35.5 ± 3.1</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>23</td>
<td>1.57</td>
<td>46.5</td>
<td>18.9</td>
<td>5260</td>
<td>7900 ± 180</td>
<td>32.5 ± 1.0</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>23</td>
<td>1.70</td>
<td>66.5</td>
<td>23.0</td>
<td>6060</td>
<td>11160 ± 130</td>
<td>36.4 ± 1.8</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>19</td>
<td>1.59</td>
<td>52.0</td>
<td>20.5</td>
<td>5080</td>
<td>9370 ± 890</td>
<td>34.3 ± 2.2</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>25</td>
<td>1.78</td>
<td>70.0</td>
<td>22.1</td>
<td>6542</td>
<td>11860 ± 120</td>
<td>36.6 ± 2.4</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>26</td>
<td>1.82</td>
<td>82.7</td>
<td>25.0</td>
<td>7168</td>
<td>13940 ± 200</td>
<td>38.6 ± 1.9</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>26</td>
<td>1.64</td>
<td>56.0</td>
<td>20.8</td>
<td>6464</td>
<td>9630 ± 220</td>
<td>35.5 ± 2.1</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>23</td>
<td>1.78</td>
<td>70.0</td>
<td>22.1</td>
<td>7338</td>
<td>11650 ± 420</td>
<td>36.9 ± 2.5</td>
</tr>
</tbody>
</table>
nitrogen content of approx. 1 g of wet homogenized faeces was determined by Kjeldahl analysis. The bacterial content of 0.5 g samples of freeze-dried faeces was determined by repeated centrifugation using a modification of the method of Stephen and Cummings [15]. The nitrogen content of faecal bacteria was determined in duplicate by Kjeldahl analysis. A sample of freeze-dried stool or the bacterial fraction (approx. 2 mg) was weighed accurately into tin capsules. A reference standard solution of ammonium sulphate (5 µl of solution containing 1 µmol/ml) was pipetted into tin capsules containing acid-washed Chromosorb (Elemental Microanalysis Ltd., Okehampton, U.K.). The samples and reference standard were analysed to determine the relative amount of excess nitrogen in urine. The proportion of the dose of administered lactose-[15N,15N]urea excreted in urine as [15N,15N]urea is given by:

$$\text{Proportion of dose of lactose-[15N,15N]urea excreted} = \frac{\text{rate of excretion of [15N,15N]urea}}{\text{+ rate of administration of lactose-[15N,15N]urea}}$$

The time taken from the ingestion of lactose-[15N,15N]urea to the first appearance of [15N,15N]urea in urine represents the minimum time needed for lactose-ureide to pass from the mouth, traverse the small intestine and be presented in the colon for hydrolysis by the microflora, and hence is a marker for oro–caecal transit time. The time taken for 14CO2 to appear on breath represents the oro–caecal transit time plus the time taken for lactose-ureide to be hydrolysed by the colonic microflora plus the time taken for urea to be hydrolysed. The cumulative excretion of 14CO2 on breath is a measure of the extent to which a single dose of lactose-ureide is fermented and hydrolysed with the release of 14CO2, calculated as:

$$\text{Cumulative excretion of } ^{14}\text{CO}_2 = \sum_{t=0}^{t=72} \text{excess } ^{14}\text{CO}_2 (\text{mmol/h})$$

The time taken from the ingestion of lactose-[15N,15N]urea to the first appearance of [15N,15N]urea in urine can be determined (Scheme 2). The excretion in stool of label was determined as:

$$\text{Proportion excreted in stool} = \frac{\text{15N molar excess } \times \text{ stool N}}{\text{total } ^{15}\text{N administered}}.$$

Of the ingested lactose-[15N,15N]ureide, a proportion of the label will be excreted in stool, a proportion will be absorbed as [15N,15N]urea and excreted in urine, and the rest will be hydrolysed to form [15N]ammonia which is...
available to metabolism. A proportion of the \( {^{15}}N \)-ammonia will re-form urea and be excreted in urine as \( {^{14}}N, {^{15}}N \)urea. Hence the proportion of the labelled urea absorbed following hydrolysis, but not retained in the system, can be determined from the rate of excretion of \( {^{15}}N, {^{14}}N \)urea. All label that is not recovered in breath, urine or stool is presumed to have been retained within the system, and the proportion of urea-nitrogen absorbed and retained, following hydrolysis, can be determined as the label not recovered in any other form:

Atoms of \( {^{15}}N \) retained

\[ = \text{atoms of } {^{15}}N \text{ ingested} \]
\[ - \text{(atoms of } {^{15}}N \text{ in stool)} \]
\[ + \text{atoms of } {^{15}}N \text{ in urine)} \]

**RESULTS**

Studies were completed satisfactorily in six women and three men, aged between 19 and 26 years (23 years on average). The subjects were on average 1.72 m tall, weighed 65 kg and had a body mass index of 21.6 kg/m\(^2\) (Table 1). The mean resting metabolic rate of the subjects was 6506 kJ/day (S.D. 781), or 102 kJ·kg\(^{-1}\)·day\(^{-1}\) (S.D. 12.9). During the course of the study, the daily energy consumption was 10993 kJ/day (S.D. 1813), equivalent to 171 kJ·kg\(^{-1}\)·day\(^{-1}\) (S.D. 4) or 1.68 (S.D. 0.19) times the resting metabolic rate. Protein consumption was 36 g/day (S.D. 1.8), or 0.57 g·kg\(^{-1}\)·day\(^{-1}\) (S.D. 0.08).

Apparent nitrogen balance was calculated as the difference between protein consumption and the sum of urinary and faecal nitrogen over a 48 h study period (Table 2). No correction was made for other unmeasured, miscellaneous losses of nitrogen from skin, or in sweat etc. All subjects were in marginally negative nitrogen balance over this period (-2.49 g of N/day), with total losses in stool and urine of 8.25 g of N/day (84% in urine and 16% in stool). Faecal nitrogen represented 23.3% (S.D. 7.6%) of nitrogen intake; of the faecal nitrogen, 22% was recovered in the bacterial fraction, with 78% in the non-bacterial fraction. There was significant recovery of \( {^{15}}N \) in stool. The level of enrichment in individual stools achieved a peak around 48 h after the initial dose of label, and had returned to background levels of abundance by 72 h. Overall, about 22% of the dose of \( {^{15}}N \) was recovered in stool, with 3% in the bacterial fraction and 19% in the supernatant fraction (Scheme 2).

Lactose-[\( ^{15}C \)]ureide was administered as a single dose, and the recovery of \( ^{15}C \) in breath \( CO_2 \) was followed for 48 h. The cumulative excretion of \( ^{15}CO_2 \) on breath is shown in Figure 1. After 48 h, 80% (S.D. 24%) of the dose administered had been recovered on breath. During the first 6 h, the recovery of \( ^{15}CO_2 \) represented less than 10% of the administered dose. The rate of recovery increased from 9 h, and by 17 h the recovery of \( ^{15}CO_2 \) was 45% (S.D. 14%) of the label administered.

Lactose-[\( ^{15}N, {^{15}}N \)]ureide was administered as prime and intermittent doses, and the recovery of label in urinary urea is shown in Figure 1. There was an increase in enrichment in urinary urea for the first 6 h, followed by a plateau level of enrichment which was maintained until the end of the study period at 18 h. Following hydrolysis of lactose-[\( ^{15}N, {^{15}}N \)]ureide, \( ^{15}N \) derived from urea might be returned to urea formation and be excreted in urine as \( {^{15}}N, {^{14}}N \)urea. Figure 1 shows the pattern of excretion of \( ^{15}N, {^{14}}N \)urea in urine with time. For the group as a whole, excretion remained at a low level for
In nine normal adults who had been consuming a low-protein diet, oral doses of lactose-[13C]ureide and lactose-[15N,15N]ureide were administered, and the recovery of label on breath (as 13CO2) and in urine (as either [15N,15N]urea or [15N,14N]urea) was determined for periods of up to 48 h. Values are means ± S.E.M. for each time point.

For each of the isotopic species, there was considerable variability among individuals, i.e. for the cumulative excretion of 13CO2 and the pattern of its recovery on the breath, and for the excretion in urine of [15N,15N]urea and [15N,14N]urea (Figure 2). When the pattern of 13CO2 on breath was compared among individuals, based on the time of peak excretion, the subjects appeared to fall into three separate groups (Figure 2). In four subjects, maximal excretion was achieved between 9 and 13 h after the dose, and in three it was between 15 and 16 h following the dose. In two subjects there were only low levels of excretion of label, with no convincing peak at any of the time points when samples were taken. Indeed,
it appeared possible that peak excretion might have occurred between 18 h and 24 h, during a period when no samples were taken. On this basis, the three groups were identified as having an early, mid or late peak of excretion of $^{13}$CO$_2$. The patterns of excretion of $^{15}$N,$^{15}$Nurea and $^{13}$N,$^{15}$Nurea in urine in these three groups are shown in Figure 2. There were no differences among the groups for the timing or pattern of enrichment in $^{15}$N,$^{15}$Nurea. For all groups, plateau enrichment was achieved by 3–6 h and was maintained until the end of the study period. However, for $^{15}$N,$^{15}$Nurea there were marked differences among the groups. Those subjects who had an early peak of $^{13}$CO$_2$ on breath had all achieved plateau levels of enrichment of $^{15}$N,$^{15}$Nurea in urine by 18 h (the end of the study period). In contrast, those who had a mid-peak in $^{13}$CO$_2$ excretion appeared to just fail to achieve plateau enrichment in $^{15}$N,$^{15}$Nurea. In contrast, for those who had a late, or no, peak in $^{13}$CO$_2$ in breath, the levels of enrichment achieved in urine for $^{15}$N,$^{15}$Nurea were very low, with no indication that they had achieved plateau enrichment within the study period.

The relative disposal of $^{15}$N derived from lactose-$^{15}$N,$^{15}$Nureide to different metabolic fates is shown in Scheme 2, based upon a reference value for ingested $^{15}$N of 100. Of ingested lactose-$^{15}$N,$^{15}$Nureide, 22% appeared in stool, with 3% in the bacterial fraction and 19% in the non-bacterial fraction. The excretion of $^{15}$N,$^{15}$Nurea indicates that, following hydrolysis of lactose-ureide, at least 5% was absorbed and excreted intact. Therefore 73% of the dose was absorbed in a form that was potentially available for metabolism, and 49% of the oral dose (or 67% of the absorbed label) was retained within the body’s pool of metabolic nitrogen. Of the dose of label, 24% (or 33% of that absorbed) was excreted in urine as $^{15}$N,$^{15}$Nurea.

**DISCUSSION**

The present study has shown that, in normal adults consuming a diet which is low in protein, oral doses of lactose-ureide are metabolized within the body. When labelled urea is administered orally, there is very rapid appearance of label on breath following hydrolysis in the stomach by *Helicobacter pylori*, or in urine following absorption of the intact molecule [16,17]. The time taken for label from lactose-ureide to appear in end-products of metabolism is consistent with the idea that sugar-ureide is resistant to digestive enzymes and is only degraded through the activity of the colonic microflora [7,9]. Following a single oral dose of lactose-$^{13}$Cureide, 80% of the label was recovered on breath as $^{13}$CO$_2$, and following oral doses of lactose-$^{15}$N,$^{15}$Nureide about 80% of the label failed to appear in stool. Together these observations suggest that, under the conditions of the present study, at least 80% of the dose administered was metabolized by the microflora. We did not determine any losses of $^{13}$CO$_2$ in urine, but other studies have suggested that this might represent 10–12% of the administered dose [8]. If label is lost in urine as the intact urea molecule, we would have expected urinary losses of $^{13}$CO$_2$ to be approx. 5% of the dose administered, based upon recovery of $^{15}$N,$^{15}$Nurea in urine (Table 3). For normal adults, consuming their habitual diet estimated to contain approx. 85 g of protein/day, the recovery of label in breath and urine only amounted to about 60% of the administered dose [8]. Although, because of the many differences between subjects and study design, it is not possible to draw a direct comparison between the studies, the suggestion that lactose-ureide might be metabolized to a greater extent in subjects consuming a low-protein diet needs formal exploration [4,10,18]. The capacity of the human duodenum or colon to hydrolyse the sugar–urea bond has been measured in material obtained at biopsy. *In vitro*, there was no evidence of any activity in either tissue [9]. These results indicate that colonic bacteria are responsible for the metabolism of orally administered lactose-ureide.

In previous studies we have explored colonic handling by placing labelled urea within the lumen of the colon, either at colonoscopy or in patients with a defunctioning colostomy [5,6]. In both these situations the colon is perturbed, but in each we found very rapid absorption of the labelled urea or the products of its hydrolysis. There were small but identifiable differences between the ascending and descending colon [5]. In the present study the approach used allows exploration of function in normal individuals without any perturbation of normal colonic function, but we cannot differentiate with any precision activity at different levels of the colon. Of the dose of lactose-$^{15}$N,$^{15}$Nureide, 22% was recovered in stool, with 3% present in bacteria. We are not able to identify the form of the label in the non-bacterial fraction, but this may in part comprise lactose-$^{15}$N,$^{15}$Nureide which escaped the action of the microflora and was excreted unchanged in the stool. If this were so, then it would mean that the available dose of labelled urea represented 80% of that administered orally. The bacterial fraction of faeces represented on average 5.5 g/day, or 24% of the dry stool weight, which is lower than would have been predicted from other studies, in which bacteria comprised up to 60% of stool weight [13,19]. There has been little work to explore the extent to which dietary and metabolic differences contribute to variability in the bacterial content of stool. The implication of the present results is of greater variability than has been appreciated, and the fact that the bacterial fraction might be reduced in subjects on a lower-protein diet requires more detailed exploration. Of the label recovered in stool, 14% was in the bacteria, and we have shown this to be in the form of bacterial protein [20].

Approx. 30% of the label ingested as lactose-
The label in urine, 5% of the dose was \(^{15}\text{N},^{15}\text{N}\)urea, i.e. urea that had been absorbed intact following hydrolysis of lactose-\(^{15}\text{N},^{15}\text{N}\)ureide. This is likely to be an underestimate of the total absorption of \(^{15}\text{N},^{15}\text{N}\)urea, as 60% might have returned to the colon and been metabolized further [4]. When \(^{15}\text{N},^{14}\text{N}\)urea is placed directly in the colon at colonoscopy, about 5% of the label is recovered intact in the urine [6]. On linear regression analysis, there was a significant correlation in the present study between the apparent absorption of label from lactose-\(^{15}\text{N},^{15}\text{N}\)ureide and the appearance of label in urine as \(^{15}\text{N},^{15}\text{N}\)urea \((r = 0.6, P < 0.01)\). Of the lactose-\(^{15}\text{N},^{15}\text{N}\)ureide given orally, 24% was recovered in urine as \(^{15}\text{N},^{14}\text{N}\)urea. This represents urea formed in the body from \(^{15}\text{N}\)ammonia, which is either absorbed as \(^{15}\text{N}\)ammonia directly from the colon or represents \(^{15}\text{N}\)ammonia or other \(^{15}\text{N}\)-labelled compounds produced from other rapidly turning over metabolite(s). The recovery of label as \(^{15}\text{N},^{14}\text{N}\)urea in urine was greater than that obtained when \(^{15}\text{N},^{15}\text{N}\)urea was placed directly in the colon [5,6]. It is likely that this difference is a reflection of the varied conditions operating within the lumen of the colon from one situation to another. In the present work, about 49% of the label derived from ingested lactose-\(^{15}\text{N},^{14}\text{N}\)ureide urea, or 67% of the label available from hydrolysed lactose-\(^{15}\text{N},^{15}\text{N}\)ureide, was retained within the body at the end of the experimental period (Scheme 2).

Previous investigators who have used lactose-ureide to probe gastrointestinal function have been concerned mainly to determine gastrointestinal transit time [7,8]. They have estimated the oro–caecal transit time as the time that elapses following oral ingestion of labelled material to the appearance on breath as labelled CO\(_2\) or in urine as labelled nitrogen. Using these criteria, in the present study we were struck by the variability in the apparent transit time among individuals, and the differences in timing of the appearance of different labels. The release of labelled nitrogen or carbon from urea is presumed to be the consequence of a two-step process (Scheme 1). In the first step, the bond between lactose and urea is broken by bacterial allantoate amidinohydrolase. In the second step the urea is hydrolysed by bacterial urease. The timing of these two steps can be determined by differentiating the appearance of the product of the first step \((^{15}\text{N},^{15}\text{N}\)urea) from that of the products of the second step \((^{13}\text{CO}_2\) or \(^{15}\text{N},^{14}\text{N}\)urea). Figures 1 and 2 show that for all subjects there was appreciable recovery of \(^{15}\text{N},^{15}\text{N}\)urea in urine from the first time point at which urine was collected (3 h), and that plateau levels of enrichment had been achieved by 6 h. From the results of the present study it is not possible to identify the earliest time at which labelled urea might have been identified in urine, but we can conclude that for each subject the minimum oro–caecal transit time was likely to be less than 3 h. This contrasts with the pattern of appearance of either \(^{13}\text{CO}_2\) in breath or \(^{15}\text{N},^{14}\text{N}\)urea in urine. Figure 2 shows that for \(^{13}\text{CO}_2\) the timing of the peak appearance in breath varied from 9 h to beyond 18 h, and further that the subjects could be divided into three broad groups based on the timing of the peak appearance of \(^{13}\text{CO}_2\) on breath. When the appearance in urine of \(^{15}\text{N},^{14}\text{N}\)urea was followed among these three groups, similar differences emerged in terms of the timing of the appearance of label. Thus, although the time taken for the bond to be broken between lactose and urea, with the release of \(^{15}\text{N},^{14}\text{N}\)urea, was similar among all individuals, the further hydrolysis of urea leading to either the release of \(^{13}\text{CO}_2\) or the formation and excretion of \(^{15}\text{N},^{14}\text{N}\)urea varied. We conclude that, although the appearance of \(^{15}\text{N},^{14}\text{N}\)urea in urine might reasonably be used to

### Table 3  Recovery of \(^{15}\text{N}\) from stool and urine following oral lactose-\(^{15}\text{N},^{15}\text{N}\)ureide

<table>
<thead>
<tr>
<th>Subject</th>
<th>Stool (^{15}\text{N})</th>
<th>Urine (^{15}\text{N},^{15}\text{N})</th>
<th>(^{15}\text{N},^{14}\text{N})</th>
<th>Total</th>
<th>Retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.99</td>
<td>18.3</td>
<td>23.6</td>
<td>5.2</td>
<td>23.0</td>
</tr>
<tr>
<td>2</td>
<td>0.27</td>
<td>1.0</td>
<td>17.8</td>
<td>8.3</td>
<td>31.7</td>
</tr>
<tr>
<td>3</td>
<td>1.41</td>
<td>5.2</td>
<td>12.7</td>
<td>4.8</td>
<td>27.0</td>
</tr>
<tr>
<td>4</td>
<td>1.14</td>
<td>7.0</td>
<td>17.7</td>
<td>6.0</td>
<td>25.8</td>
</tr>
<tr>
<td>5</td>
<td>1.32</td>
<td>30.2</td>
<td>34.7</td>
<td>5.4</td>
<td>7.0</td>
</tr>
<tr>
<td>6</td>
<td>5.15</td>
<td>10.9</td>
<td>28.8</td>
<td>5.0</td>
<td>25.6</td>
</tr>
<tr>
<td>7</td>
<td>5.13</td>
<td>19.8</td>
<td>25.0</td>
<td>3.5</td>
<td>18.2</td>
</tr>
<tr>
<td>8</td>
<td>0.86</td>
<td>6.9</td>
<td>21.8</td>
<td>4.6</td>
<td>42.1</td>
</tr>
<tr>
<td>9</td>
<td>0.08</td>
<td>12.0</td>
<td>16.0</td>
<td>6.1</td>
<td>16.8</td>
</tr>
<tr>
<td>Mean</td>
<td>2.04</td>
<td>12.37</td>
<td>22.0</td>
<td>5.4</td>
<td>24.1</td>
</tr>
<tr>
<td>S.D.</td>
<td>1.94</td>
<td>9.01</td>
<td>6.9</td>
<td>1.3</td>
<td>9.9</td>
</tr>
</tbody>
</table>

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estimate oro–caecal transit time, this was not necessarily true for the appearance of $^{14}$CO$_2$ on breath or of [15N,14N]urea in urine. The subjects studied by Wutzke et al. [8] were given lactose-ureide for 24 h (1 g every 4 h) before the study with labelled material was carried out. The effect was to shorten the time taken for label to appear on breath or in urine, and the overall excretion of label was increased [8]. These authors suggested that these observations support the idea that priming with a suitable substrate is needed in order to induce bacteria with the necessary ability to ferment lactose-ureide. This suggests that differences in the presence and activity of bacteria with the necessary enzyme systems might account for some of the variability in the results of the present study. However, it is also possible that the differences in timing between the groups of subjects mark different bacterial activity at different lengths along the gastrointestinal tract. If this were so, then the reliability of using the recovery of $^{14}$CO$_2$ or [15N,14N]-urea to assess oro–caecal transit time is called into doubt and needs to be re-assessed.

The present study has shown that lactose-ureide can be used to obtain information on aspects of the handling of material by the colonic microflora in normal human subjects. There appear to be differences in the metabolic activity of the microflora, which may in part be related to differences in the amount of protein taken in the diet by the host. There is considerable variability among individuals, and a definition of the basis of this variability is likely to make a useful contribution to our understanding of the behaviour of this complex ecosystem.

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REFERENCES


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