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# Validation of lactose[$^{15}$N,$^{15}$N]ureide as a tool to study colonic nitrogen metabolism

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**Geboes, Karen P., Vicky De Preter, Anja Luypaerts, Bert Bammens, Pieter Evenepoel, Yvo Ghoos, Paul Rutgeerts, and Kristin Verbeke.** Validation of lactose[$^{15}$N,$^{15}$N]ureide as a tool to study colonic nitrogen metabolism. *Am J Physiol Gastrointest Liver Physiol* 288: G994–G999, 2005. First published December 30, 2004; doi:10.1152/ajpgi.00408.2004.—In vitro experiments have shown that fermentation of carbohydrates prevents accumulation of nitrogen in the colon. Variable results have been obtained on modulation of dietary intakes in vivo. Lactose[$^{15}$N,$^{15}$N]-labeled ureide has been proposed as a tool to study colonic nitrogen metabolism. However, on oral administration of the marker, different urinary excretion patterns of the $^{15}$N label have been found. In this study, 50 mg lactose[$^{15}$N,$^{15}$N]ureide was directly instilled in the colon through an orocecal tube to investigate the colonic handling of this molecule in a direct way. In basal conditions, 42% (range, 37–48%) of labeled nitrogen administered as lactose[$^{15}$N,$^{15}$N]ureide was retrieved in urine after 72 h. A substantial variability in total urinary excretion of the label was found, but the urinary excretion pattern of the label was similar in all volunteers. When inulin, a fermentable carbohydrate, was administered together with the labeled marker, a significant decrease in urinary excretion of $^{15}$N after 72 h was found, to 29% (range, 23–34%). The effect of a smaller dose of inulin (250 mg) on colonic handling of lactose[$^{15}$N,$^{15}$N]ureide (50 mg), was investigated in another group of volunteers, and this time, fecal excretion of the marker was also evaluated. The results seem to indicate that fermentation of inulin causes an increased fecal excretion of the marker, thereby reducing urinary excretion but not retention in the human nitrogen pool. This instillation study shows that lactose[$^{15}$N,$^{15}$N]ureide is a tool with good properties to investigate the effect of different types of carbohydrates on nitrogen metabolism in the proximal colon in vivo.

carbohydrate fermentation; stable isotopes

THE ACCUMULATION OF AMMONIA in the colon is of great importance for human health. First, part of the ammonia formed in the colon is salvaged for human metabolism. On a normal protein diet, it has been estimated that every day ~30% of the nitrogen present in urea produced in the liver is salvaged from the colon and 70% of the salvaged nitrogen is utilized for amino acid and protein synthesis (7). Second, the amount of ammonia produced in the colon is important in some pathological conditions, such as chronic portosystemic encephalopathy and end-stage renal failure (3, 26). Third, some of the effects of ammonia described in in vitro studies may point to a possible local toxic effect on the colonic mucosa. Ammonia has been shown to affect the intermediary metabolism and DNA synthesis in colonic epithelial cells and to reduce their life span. It increases the turnover of epithelial cells, and in this way, increases the probability of genetic damage occurring in the presence of oncogenic agents because dividing cell populations are more susceptible to chemical carcinogenesis (22).

Ammonia is derived from proteolysis and ureolysis in the lumen of the large intestine. On average, 0.3–4.1 g of nitrogen, the majority in the form of protein and peptides, enter the colon daily from the small bowel (11). The polypeptide chains in dietary and endogenous protein are hydrolyzed by proteases and peptidases to amino acids, which are further metabolized through various reactions involving deamination with the production of ammonia. Ammonia can also be derived from urea. There are two possible ways in which urea may reach the microflora of the large bowel: through the ileocecal valve or by diffusion of blood urea in intestinal contents. The flux of urea from the ileum to the colon is estimated to be only 0.75 g/day (5). The flux of urea by diffusion from the blood through the colonic wall depends on protein intake, metabolic state of the host, and energy supply to the colonic flora. Intraluminal hydrolysis of urea into ammonia is so fast that very little intact urea molecules may be detected in the colonic lumen.

The accumulation of ammonia in the large bowel can be decreased by reducing the dietary protein intake, but also by administration of fermentable carbohydrates. In vitro experiments have shown that carbohydrate fermentation affects bacterial protein metabolism in different ways. Carbohydrate fermentation results in a decrease of the pH through production of short-chain fatty acids. As a consequence, protease activity will decrease because large intestinal proteases have a neutral to alkaline pH optimum (11, 19). In addition, amino acid metabolism is decreased when fermentable carbohydrates are present in the medium (18, 19, 21, 23). This effect is suggested to be caused by catabolite repression, which implies that in the presence of a certain substrate, in this case fermentable carbohydrates, the transcription of the genes involved in the bacterial utilization of another substrate (amino acids) is repressed, and their corresponding products are inactivated and degraded. Both mechanisms lead to a decreased production of ammonia. On the other hand, fermentation of carbohydrates provides an energy source to the microflora, thereby expanding bacterial mass and increasing the incorporation of ammonia and amino acid nitrogen into the bacteria (20). It is not clear whether colonic bacteria prefer free luminal amino acids or ammonia as nitrogen source (6, 27).

Until now, all studies concerning colonic nitrogen metabolism involving human volunteers have been performed by measurement of nitrogen excretion in urine and feces. These

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studies led to contradictory results (1, 2, 9, 24, 25, 29). These differences could be attributed to the fact that a variety of factors are implied in the regulation of colonic nitrogen metabolism, including protein intake in the diet and the metabolic state of the host. Within the large bowel, differences in fermentation processes have been described in the different regions. Carbohydrate fermentation occurs mainly in the proximal colon (12). On exhaustion of fermentable carbohydrates, extensive proteolysis may still occur in the distal colon, suggesting that fermentation of carbohydrates causes merely a delay in protein fermentation, especially on high protein intakes (9). Nutrient availability and especially the ratio of available carbohydrates to nitrogen, depending both on the amount and type of protein and fermentable carbohydrate taken in the diet, may be the most important factors controlling nitrogen metabolism in vivo (10, 17, 29). Fermentation of carbohydrates influences both protein degradation and nitrogen uptake by bacteria.

The use of stable isotopes is a safe and elegant way to investigate physiological processes in vivo. In this study, lactose[$^{15}$N,$^{15}$N] ureide was used as a source of labeled nitrogen to study the fate of colonic urea-nitrogen in the proximal colon *1*) in normal conditions and *2*) on administration of fermentable carbohydrates.

On oral administration, lactose-ureide reaches the colon unmodified, because the molecular bond between the carbohydrate moiety and urea in lactose ureide has been shown to resist enzymatic degradation in the human gastrointestinal tract (13). When lactose[$^{15}$N,$^{15}$N]ureide reaches the colon, it is degraded to [$^{15}$N,$^{15}$N]-labeled urea by selected bacteria. The labeled urea undergoes fast hydrolysis with production of $^{15}NH_3$ (28). The labeled $NH_3$ is mixed with the ammonia present in the colon, and as a consequence, the variations observed in the $^{15}$N-labeled measurement reflect the fate of total colonic $NH_3$.

However, in this study, lactose-ureide was not administered orally but directly introduced into the colon to avoid confounding differences in orocecal transit time. The substrate was administered through instillation in order not to disturb the normal environment and physiology of the colon. In the past, studies concerning colonic nitrogen metabolism in vivo have been performed on patients with a colostomy or by accessing the lumen through colonoscopy (14, 15). As opposed to the method used in this study, neither of these approaches can be used to assess normal function. People with a colostomy have suffered from colonic disease and bowel preparation before colonoscopy deranges the normal colonic environment. Inulin was chosen to investigate the effect of carbohydrate fermentation on the excretion of colonic nitrogen (4, 16).

## SUBJECTS AND METHODS

*Experimental design.* The study was carried out in 14 healthy volunteers (6 men, 8 women). Each of them performed the experiment twice, once without inulin and once with inulin, with an interval of at least 1 wk. The volunteers kept diet records the week before each experiment to allow a qualitative comparison of dietary intakes. They were allowed to eat their usual diets, but were urged to keep a constant macronutrient composition.

On the day before the test, each subject swallowed a narrow polyvinyl tube (bowel decompression catheter, 5.3 mm; Rusch Manufacturing, Lurgan, Armagh, N. Ireland). The position of the tube was followed by fluoroscopy. When the tip of the tube had reached the ligament of Treitz, the volunteer was allowed to return home and eat, whereupon the tube progressed down the intestine. All subjects ingested 1 g of unlabeled lactose ureide that evening and then fasted overnight.

The next morning, brief radiographic screening was performed to ensure that the tip of the tube was in the right colon. The labeled substrates were dissolved in 40 ml NaCl 0.9% and injected in bolus. The tube was flushed with another 40 ml NaCl 0.9%. Thereafter, the tube was removed. The volunteers were not allowed to eat or drink during the first 4 h of the test. After 4 h, they received a ham or cheese sandwich.

Nine of the volunteers performed a 3-day urine collection on each test occasion. On their first visit, a 40-ml solution containing 50 mg lactose[$^{15}$N,$^{15}$N]ureide was administered. Two grams of inulin were added to the infusion in the second experiment.

The other five volunteers performed a 3-day urine and stool collection in the two experimental conditions. They received a 40-ml solution containing 50 mg lactose[$^{15}$N,$^{15}$N]ureide and 185 kBq of $^{3}$H-labeled polyethylene glycol ($^{3}$H-PEG) on their first visit and the same solution with an additionally 250 mg inulin on the second visit.

*Substrates.* Fifty milligram of lactose[$^{15}$N,$^{15}$N]ureide, synthesized according to the method of Schoorl as modified by Hofmann (28) with [$^{15}$N,$^{15}$N]urea obtained from Euriso-top, Saint-Aubin, Cédex, France, were administered directly into the colon. One gram of unlabeled lactose ureide was ingested on the evening before the test to induce the proper enzyme activity in the colonic bacteria (8, 28).

Raftilin high performance (HP) (Orafti, Tienen, Belgium), a linear β(2,1)-linked fructose polymer, terminated by a sucrose residue purified from chicory root was used as a prototype for a fermentable carbohydrate. Raftilin HP contains >99% inulin with a degree of polymerization ranging between 5 and 60 and <0.5% glucose, fructose, and sucrose.

Whenever correction for transit time was required, 185 kBq of $^{3}$H-PEG (New England Nuclear Life Science Products, Boston, MA) were added to the infusion as an inert radiolabeled transit marker.

*Sample collection.* Urine samples were collected in plastic containers to which 1 gram of neomycin was added to prevent bacterial growth. All volunteers were asked to void before the infusion, and this urine collection was used for measurement of basal nitrogen content and $^{15}$N enrichment. After the infusion, urine was collected over 3 consecutive days in different fractions: 0–3 h, 3–6 h, 6–9 h, 9–24 h, 24–48 h, and 48–72 h. After measurement of the volume, samples were taken and stored at −20°C until analysis.

Five volunteers also performed a stool collection at 72 h. Date and time of voiding of stools were noted in a diary. The stools were frozen immediately after voiding, weighed, and then stored at −20°C. All stools collected on the same day were combined and homogenized before further analysis. Samples of known weight were removed and freeze-dried. The dried material was weighed again, and aliquots were taken for analysis of nitrogen and radioactivity.

*Analyses.* Total N content and $^{15}$N enrichment of urine and feces were measured by using an elemental analyzer (ANCA-SL; PDZ, Cheshire, UK) coupled with both a thermal conductor detector (TCD; PDZ) and a stable isotope ratio mass spectrometer (IRMS; PDZ). A known volume of urine (15 μl) or feces (~7 mg) was oxidized in the presence of oxygen at 1,000°C. The combustion products thereafter passed through a second furnace containing copper at 600°C where excess oxygen was absorbed and nitrogen oxides were reduced to elemental nitrogen. Total nitrogen content was measured by means of a TCD, whereas the $^{15}$N enrichment was determined by means of an IRMS detector, coupled to the combustion unit of the elemental analyzer. The aim of this validation study was the evaluation of the cumulative urinary and fecal excretion of the label and the urinary excretion pattern of the label and not the identification of the molecules in which the labeled nitrogen was retrieved.

Creatinine and urea content in urine were measured by standard laboratory techniques. The $^{3}$H-PEG content in stool was measured

with liquid-scintillation counting (Tricarb liquid scintillation spectrometer, model 3375; Packard Instruments, Downers Grove, IL) after oxidation to $^{3}H$-$H_2O$ (Packard sample oxidizer, model 306).

For each urine collection, the results were expressed as total nitrogen (grams), urea (grams) and creatinine (grams), and percentage of administered dose $^{15}$N recovered. A correction for creatinine content was performed to correct for mistakes due to dilution or differences in sampling times, which may have occurred in the first short collection periods (grams urea/grams creatinine and micrograms $^{15}$N/grams creatinine).

Both for urinary and fecal measurements, total nitrogen (gram) excretion and cumulative percentage of administered dose $^{15}$N recovered over 24/48/72 h were evaluated. Tritium contents in stool were expressed as percentage of administered dose recovered over 72 h and were used to correct for gastrointestinal transit by dividing the cumulative percentage of administered dose $^{15}$N recovered over 72 h by the cumulative percentage of administered dose of $^{3}$H recovered over 72 h.

*Statistical analysis.* Because of the low number of measurements, all results were expressed as median plus interquartile range, and nonparametric statistical analysis was used. Alpha was set at 0.05. (Wilcoxon test, Mann-Whitney *U*-test, Statistica 6.0, and Statsoft 1984–2001).

## RESULTS

*Urinary total nitrogen excretion and kinetics of urinary $^{15}$N excretion in normal conditions (n = 14).* The urinary excretion of labeled nitrogen is presented as a function of time in Fig. 1. The excretion rate of labeled nitrogen was maximal between 3 and 9 h after instillation in all volunteers. The $^{15}$N recovery in urine had returned to baseline by the end of the test period, indicating that the urinary excretion of the label was completed. The cumulative urinary excretion of $^{15}$N increased up to 28% (range, 22–38%) of the administered dose at 24 h and 45% (range, 34–51%) at 72 h. Total nitrogen excretion in urine varied ~10 g/24 h. A diurnal variation in urinary nitrogen and urea excretion was noted.

*Effect of instillation of 2 g of Raftilin HP on urinary excretion of total nitrogen and $^{15}$N (n = 9).* The results are summarized in Table 1. No difference in urinary excretion of total nitrogen was noted; urinary nitrogen excretion varied ~10 g/24 h in all the collections. No difference in total urinary creatinine or urea excretion was measured either. No differences in diurnal variation of urinary nitrogen and urea excretion were noted after correction for creatinine content (Fig. 2).

Table 1. *Total nitrogen and $^{15}$N excretion in urine after colonic instillation in healthy volunteers of lactose[$^{15}$N,$^{15}$N]ureide with and without 2 g Raftilin HP*

| | Baseline Conditions | Addition of 2 g Inulin |
|---|---|---|
| Urinary nitrogen excretion (g/72 h) | 30 (25–31) | 26 (18–30) |
| Urinary urea excretion in the first 24 h (g) | 21 (15–29) | 17 (14–22) |
| Total urinary urea excretion (g/72 h) | 54 (38–66) | 45 (30–51) |
| Urinary creatinine excretion in the first 24 h (g) | 0.8 (0.7–1.3) | 0.9 (0.8–1.1) |
| Cum%/24 h of $^{15}$N recovered in urine (%) | 27 (23–30) | 20 (18–21)* |
| Cum%/72 h of $^{15}$N recovered in urine (%) | 42 (37–48) | 29 (23–34)* |

Values are median ± interquartile range (IQR); numbers in parentheses are ranges; $n = 9$. *Value differs from baseline conditions, $P < 0.02$. HP, high performance.

A consistently lower excretion rate of labeled nitrogen on administration of inulin was noted in the first two urine collections, leading to a statistically significant reduction in cumulative urinary excretion of labeled nitrogen at 24 h; 27% (23–30%) of administered dose was retrieved in urine in normal conditions vs. 20% (18–21%) after addition of 2 g of Raftilin HP to the infusion (Fig. 3) (Wilcoxon test, $P = 0.01$). The statistically significantly lower recovery of the label was still noted 72 h after the instillation (Wilcoxon test, $P = 0.02$).

After correction for creatinine content, a statistically significant decrease in excretion rate of the marker was observed in the 3–6 h collection [15 μg (range, 11–22 μg) $^{15}$N/g creatinine vs. 11 μg (range, 6–14 μg $^{15}$N/g creatinine; Wilcoxon test, $P = 0.03$] (Fig. 4).

*Effect of colonic instillation of 250 mg of Raftilin HP on urinary parameters (n = 5).* The results are summarized in Table 2. Five volunteers performed a 3-day urine collection after colonic instillation of 50 mg lactose[$^{15}$N,$^{15}$N]ureide and once again after simultaneous administration of lactose[$^{15}$N,$^{15}$N]ureide and 250 mg Raftilin HP. The urinary total nitrogen excretion remained unchanged, varying ~10 g/24 h in all 24-h collections with and without 250 mg Raftilin HP.

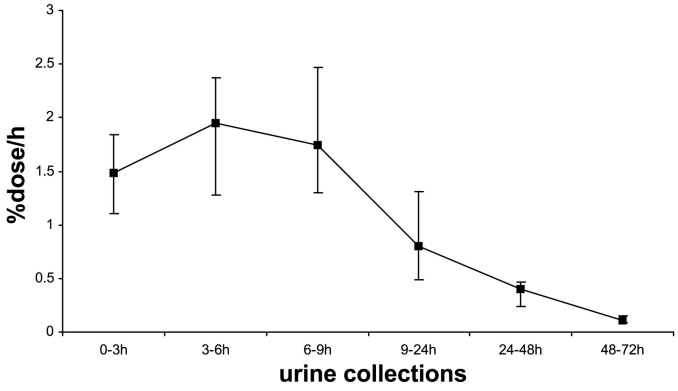


Fig. 1. Urinary excretion pattern of labeled nitrogen expressed as percentage of administered dose recovered per hour, on colonic instillation of lactose[$^{15}$N,$^{15}$N]ureide in normal circumstances in healthy volunteers. Values are median ± interquartile range (IQR), $n = 14$.

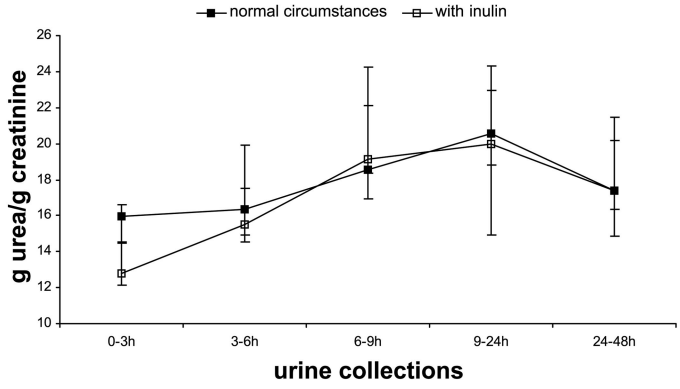


Fig. 2. Diurnal variation in urinary urea excretion (expressed as g urea/g creatinine) in normal circumstances and when 2 g Raftilin HP are administered through colonic instillation in healthy volunteers. Values are median ± IQR, $n = 9$.

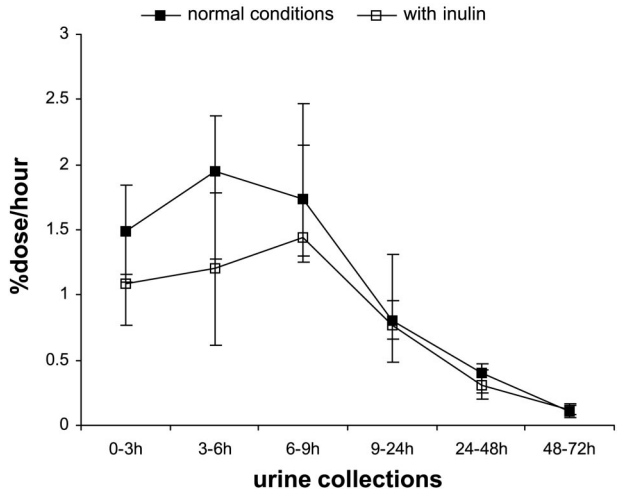


Fig. 3. Urinary excretion pattern of labeled nitrogen (expressed as %administered dose recovered per hour) on colonic instillation of lactose[$^{15}$N,$^{15}$N]ureide in healthy volunteers, in normal circumstances and when 2 g Raftilin HP are administered simultaneously. Values are median ± IQR, $n = 9$.

A clear reduction in cumulative urinary excretion of labeled nitrogen was observed after simultaneous administration of inulin and was mainly due to a lower excretion rate of labeled nitrogen in the first two urine collections. However, statistical significance was never obtained.

*Effect of amount of Raftilin HP on changes in cumulative urinary $^{15}$N excretion.* Both doses of Raftilin HP caused a similar absolute reduction in cumulative urinary excretion of the marker: 12% (range, 8–16%) reduction after administration of 250 mg of inulin and 7% (range, 4–19%) after administration of 2 g of inulin [Mann-Whitney *U*-test, $P$ = not significant (NS)]. This indicates that an amount of 250 mg of inulin is sufficient to influence the excretion route of ammonia derived from 50 mg lactose-ureide.

*Effect of colonic instillation of 250 mg of Raftilin HP on fecal parameters ($n = 5$).* The results are summarized in Table 2. The colonic transit time was not different in the two test situations: 49% (range, 35–60%) of the administered dose of $^{3}$H-PEG was retrieved after 72 h in basal circumstances and 60% (range, 22–65%) when inulin was added to the solution.

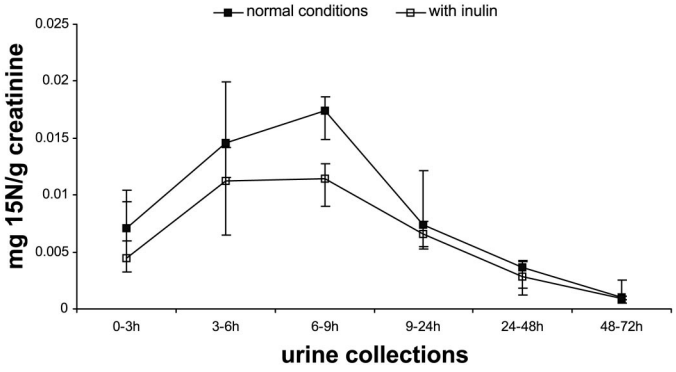


Fig. 4. Urinary excretion pattern of labeled nitrogen on colonic instillation of lactose[$^{15}$N,$^{15}$N]ureide in healthy volunteers, in normal circumstances and when 2 g Raftilin HP is administered simultaneously after correction for creatinine content (expressed as mg $^{15}$N/g creatinine). Values are median ± IQR, $n = 9$.

Table 2. *Total nitrogen and $^{15}$N excretion in urine and feces after instillation of lactose[$^{15}$N,$^{15}$N]ureide in healthy volunteers with and without 250 mg Raftilin HP*

| | Baseline Conditions | Addition of 250 mg Inulin |
|---|---|---|
| Urinary nitrogen excretion (g/72 h) | 25 (18–41) | 25 (19–30) |
| Cum%/72 h of $^{15}$N recovered in urine (%) | 46 (30–55) | 39 (37–40) |
| Colon transit time: Cum%/72 h of $^{3}$H-PEG (%) | 49 (35–60) | 60 (22–65) |
| Fecal nitrogen excretion (g/72 h) | 3.7 (1.5–5.8) | 3 (1.8–5.1) |
| Cum%/72 h of $^{15}$N recovered in feces (%) | 13 (6–18) | 16 (15–25) |
| Corrected fecal excretion of labeled nitrogen (%) | 25 (13–38) | 39 (26–49) |
| Fecal dry weight/3 days (g) | 250 (125–350) | 194 (132–386) |
| Nitrogen density (% dry matter) | 4.5 (4.1–4.9) | 4.7 (4.4–4.8) |
| Fecal density of the label (% dry matter) | 0.006 (0.004–0.009) | 0.007 (0.005–0.012) |
| Cum%/72 h of $^{15}$N recovered in feces and urine | 62 (48–75) | 64 (54–70) |

Values are median ± IQR; numbers in parentheses are ranges; $n = 5$. Cum%, cumulative percent.

No difference in total fecal nitrogen excretion after 3 days was measured either.

On the other hand, there was a clear but not statistically significant increase in fecal excretion of labeled nitrogen after 72 h; 13% (range, 6–18%) of administered $^{15}$N was excreted in normal conditions vs. 16% (range, 15–25%) after addition of 250 mg of Raftilin HP to the infusion.

No difference in fecal dry weight or in total nitrogen density was observed, but a consistent increase in fecal density of labeled nitrogen was withheld (Wilcoxon test, $P$ = NS).

*Effect of 250 mg of Raftilin HP on total excretion of the label ($n = 5$).* No difference in total cumulative excretion of the marker was measured: 62% (range, 48–75%) in the first part of the study vs. 64% (range, 54–70%) in the second, indicating no difference in retention of the marker [retention = 100 − (urinary + fecal excretion)].

Complete stool collections on both occasions were obtained in four of five volunteers. An inverse relationship between the changes in urinary and fecal parameters was found consistently.

## DISCUSSION

In this study, lactose[$^{15}$N,$^{15}$N]ureide was directly instilled in the colon through an orocecal tube to investigate colonic handling of ammonia nitrogen. A low interindividual variability in urinary excretion pattern of the label was found. Simultaneous administration of inulin, a fermentable carbohydrate, caused a consistent decrease in urinary excretion of $^{15}$N.

Due to the onerous protocol, the combined study of fecal and urinary parameters was performed in a limited number of volunteers. The observations in this study suggest that the decrease in urinary excretion of the marker is accompanied by an increase in its fecal excretion.

After administration of labeled lactose ureide, part of the label is excreted in urine. The form of $^{15}$N in urine can be either

[$^{15}$N,$^{15}$N]urea, which has escaped hydrolysis in the colon or [$^{15}$N,$^{14}$N]urea when [$^{15}$N,$^{15}$N]urea is hydrolyzed in the colon to $^{15}NH_3$, which is subsequently absorbed and converted to [$^{15}$N,$^{14}$N]urea in the liver (14, 15, 28). The techniques used in this study do not enable differentiation between [$^{15}$N,$^{15}$N]urea and [$^{15}$N,$^{14}$N]urea.

However, literature data indicate that when [$^{15}$N,$^{15}$N]urea is administered directly in the colon at colonoscopy, ~5% of the label is recovered intact in urine (15). In our study, 28% of the labeled nitrogen was recovered after instillation of 50 mg lactose[$^{15}$N,$^{15}$N]ureide. Assuming 5% of the label recovered in urine to be in the form of [$^{15}$N,$^{15}$N]urea, the remaining 23% may be considered to be derived from [$^{15}$N,$^{14}$N]urea. This figure correlates with the 24% of administered label that was recovered as [$^{15}$N,$^{14}$N]urea in urine on oral administration of labeled lactose-ureide (28).

Most importantly, the results of our study show that the urinary excretion pattern of $^{15}$N was uniform in all volunteers. In a previously performed study (12), three different urinary excretion patterns of $^{15}$N have been described on oral administration of lactose[$^{15}$N,$^{15}$N]ureide. The variability in the results was assumed to refer to differences in bacterial activity in different regions of the colon. However, our study showed a similar excretion pattern of the label in all volunteers on instillation of lactose[$^{15}$N,$^{15}$N]ureide in the right colon. This indicates that there is no major interindividual variation in degradation of the labeled lactose ureide or in hydrolysis of the formed urea once lactose-ureide has reached the colon. Therefore, we suggest that the variation in urinary excretion pattern of $^{15}$N observed on oral ingestion of labeled lactose ureide may refer to differences in orocecal transit and hence, variability in arrival of the molecule in the cecum. This implicates that it is possible to study colonic nitrogen metabolism on oral administration of lactose[$^{15}$N,$^{15}$N]ureide.

To evaluate the influence of carbohydrate fermentation on nitrogen metabolism in the colon, Raftilin HP was added to the infusion in a second experiment. The most important observation was a decrease in urinary excretion of labeled nitrogen compared with baseline values after administration of two different doses of Raftilin HP in all volunteers but one. Both doses of Raftilin HP caused a similar reduction in cumulative urinary excretion of the marker and similar changes in the excretion pattern, suggesting that a dose of 250 mg Raftilin HP is sufficient to influence the excretion route of ammonia derived from 50 mg lactose-ureide. An amount of 250 mg of inulin is extremely low compared with the colonic dietary carbohydrate load and it may appear surprising that this dose of Raftilin HP can provoke measurable effects on the excretion of labeled nitrogen. However, the reproducibility of the results urges us to attribute this effect to the administration of inulin. Both substrates are administered simultaneously and therefore are more likely to influence each other's metabolism.

Reduction in cumulative urinary excretion of the label was caused by a clear reduction in urinary excretion rate of the $^{15}$N isotope in the first hours of the test. Temporary changes in bacterial activity caused by fermentation of carbohydrates would have resulted in a change of urinary excretion pattern of the label and not necessarily in a reduction of total urinary excretion of the marker. This means that this result is suggestive for a decrease in colonic absorption of ammonia due to an increased removal of nitrogen.

Reduction in urinary excretion of the label may have been caused by changes in the bacterial or in the human metabolism. To exclude an increased retention of the label in the human nitrogen pool as a cause of the decrease in urinary excretion, the recovery of labeled nitrogen in feces was measured in five of the volunteers. The retention of the label in the human body was defined as 100% − (%dose of $^{15}$N excreted in urine + %dose of $^{15}$N excreted in feces). From these results, it was clear that the retention of salvaged nitrogen for human metabolism after administration of Raftilin HP (36%) was not different from the retention in baseline conditions (38%) and that the decrease in urinary excretion of the label was accompanied by an increase in fecal excretion. A consistent negative relationship between fecal and urinary excretion of the $^{15}$N isotope was found, suggesting a shift from urinary toward fecal excretion on carbohydrate fermentation.

Changes in urinary and fecal excretion of the marker were not caused by changes in colonic transit, because no difference was seen in the recovery of $^{3}$H-PEG in feces. The consistent increase in fecal $^{15}$N density strongly suggests an increased incorporation of $^{15}NH_3$ into the bacteria.

The combined study of fecal and urinary parameters was performed in a limited number of volunteers because of the onerous protocol, leading to restricted power. However, the marker can be administered in an oral testmeal, because the molecule can only be broken down by bacterial enzymes and no interindividual variability in urinary excretion pattern of the label was found on colonic instillation in baseline conditions. Oral administration of the marker will allow us to perform studies considering the relation between urinary and fecal excretion of colonic ammonia and the effect of different fermentable carbohydrates hereon in larger cohorts.

Results of this study indicate that lactose[$^{15}$N,$^{15}$N] ureide seems a very valuable tool to investigate the effect of different types of carbohydrates on urea-nitrogen metabolism in the proximal colon in vivo.

We conclude that colonic metabolism of lactose-ureide is rather uniform when a priming dose of lactose-ureide has been given. Although we only examined urinary and fecal excretion of the marker simultaneously in a small group, we speculate that fermentation of carbohydrates shifts colonic ammonia toward bacterial metabolism, resulting in a reduced absorption of colonic ammonia, given the consistent negative relationship between urinary and fecal excretion of the label and the statistically significant decrease in urinary excretion on administration of inulin. Fermentation of carbohydrates does not influence the amount of nitrogen used for human metabolism. Further studies based on oral administration of labeled lactose-ureide are warranted.

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