15N-urea metabolism in the functioning human colon: luminal hydrolysis and mucosal permeability

B J Moran, A A Jackson

Abstract

The biopsy channel of the colonoscope was used in a novel approach to the study of *in vivo* colonic nitrogen metabolism in 12 subjects. A tracer dose of 15N15N-urea was placed in the caecum in six and distal to the splenic flexure in six. The urine and stool were collected for 72 hours and isotopic enrichment was measured in a mass spectrometer. A similar proportion of the dose was recovered in the urine as 15N15N-urea from the right colon, 6%, as was recovered from the left, 4%, showing that the urea was absorbed intact. Urinary 15N14N-urea from the right colon was 18% of the dose compared to 13% from the left colon. This represents urea that has been hydrolysed and absorbed as ammonia. Less than 4% of the dose was recovered in the stool. The greatest proportion of the label, 74% from the right and 82% from the left, could not be accounted for in the urine or the stool and is presumed to have entered the metabolic pool of nitrogen. We conclude that; the colon is permeable to urea, intraluminal hydrolysis occurs and that urea nitrogen enters the metabolic pool of nitrogen in functionally significant quantities.

The mechanism of adaptation to low protein intakes involves the retention and salvaging of urea nitrogen, which is hydrolysed in the lower bowel rather than being excreted in the urine.¹ The metabolic handling of nitrogen in the bowel has direct relevance to a number of clinical situations, most obviously hepatic failure with portosystemic encephalopathy, and chronic renal failure.

Our understanding of the metabolism of urea by the human colon has been dominated by the observations of Wolpert *et al.*² and Bown *et al.*³ Wolpert *et al.*² using a steady state perfusion of the intact colon, found that only 2% of plasma urea was recovered from the lumen of the colon, whereas 5% of urea perfused into the colon could be accounted for by absorption. They concluded that trivial amounts of urea are excreted and hydrolysed in the lumen; the colon is effectively impermeable to urea, and that any hydrolysis that takes place is likely to be juxtamucosal rather than luminal. Bown *et al.*³ perfused the excluded colon at 10 ml/min, were unable to show either luminal or juxtamucosal hydrolysis and found limited permeability of the colonic mucosa to urea.

Since the earlier work of Walser and Bodenlos⁴ it had been clear that in the normal adult only about 70% of the urea production is excreted in the urine, with the other 30% being hydrolysed and a proportion of this being retained by the body. There is substantial evidence showing that

the retained urea is hydrolysed in the large bowel by the microflora.⁵ There are two possible ways in which urea might reach the microflora; through the ileocaecal valve or by passing through the colonic wall. Chadwick *et al.*⁶ measured the flux of urea from the ileum to the colon as 0.35 g urea nitrogen per day, only a small proportion of the daily breakdown in the colon. They concluded that substantial quantities must be excreted directly into the colon from the blood. Gibson *et al.*⁷ were able to show that the 0.39 g urea nitrogen passing through the ileocaecal valve was only a fraction of the daily ureolysis, 2.9 to 5.1 g nitrogen per day. They formed the opinion that only a minor part of the ureolysis took place in the colonic lumen. Wrong *et al.*⁸ used 15N15N labelled urea to measure the rate of urea synthesis, and hydrolysis, and based on the relative enrichment of faecal nitrogen, they also concluded that the lumen of the large bowel is not the main site of endogenous urea hydrolysis.

The findings of these studies conflict with the practical experience that modulating the metabolic activity of the gastrointestinal microflora, by the use of antibiotics, or the provision of energy substrate in the form of non-digestible, fermentable carbohydrate is a central factor in the alleviation of hepatic encephalopathy.⁹ In more recent studies we have found that, in fact, urea nitrogen traces the movement of a far larger pool of nitrogen through the bowel, of the order of 16 g nitrogen/day.¹⁰ This would explain the effective dilution of label from urea nitrogen in total faecal nitrogen found by Wrong *et al.*⁸ It is possible to quantify the movement of urea nitrogen through the colon, in relation to the intake of amino acids and the production and excretion of urea nitrogen as outlined in Figure 1. Jackson *et al.*¹¹ have shown that, on a normal protein intake of 14 g nitrogen, the daily production of urea is 9.7 g urea nitrogen and that 3 g of this is hydrolysed in the colon. This extensive exchange of nitrogen may be of considerable functional significance,¹² making it important to re-evaluate the physiological handling of nitrogen in the lower bowel.

It has been shown in animals that the permeability characteristics of the gut are enhanced by normal intestinal contents.¹³ If this were so in man, then the relatively fast flow with perfusion techniques could disturb the normal balance between the mucosa and the lumen. The use of the colonoscope offers an opportunity for investigating the colon which may approach the normal physiology more closely. In this study we have taken advantage of this technique to follow the fate of labelled urea placed in the colon at colonoscopy.

Urea contains two atoms of nitrogen, both of

Departments of Surgery and Human Nutrition, Southampton General Hospital, Southampton
B J Moran
A A Jackson

Correspondence to: Prof A A Jackson, Department of Human Nutrition, University of Southampton, Bassett Crescent East, Southampton SO9 3TU.

Accepted for publication 13 June 1989

which can be labelled with ^{15}N , to give $^{15}\text{N}^{15}\text{N}$ -urea or ^{30}N -urea. If a labelled urea molecule is absorbed intact from the colon then ^{30}N -urea will appear in the urine. Any urea that is hydrolysed in the bowel will give rise to ^{15}N labelled ammonia, which may be absorbed and reincorporated into urea, containing one labelled and one unlabelled nitrogen, $^{15}\text{N}^{14}\text{N}$ -urea, or ^{29}N -urea. The appearance of ^{29}N -urea gives a measure of the extent of colonic urea hydrolysis. The nitrogen from hydrolysed urea may be utilised for the metabolic activity of the colonic microflora or of the host, and it has been presumed that label that cannot be accounted for in urine and stool has been utilised for synthetic activities.

We have been able to show that the colon is permeable to urea, and that a substantial proportion of the urea nitrogen placed in the lumen of the colon can be retained by the host. These data imply that the metabolism of nitrogen in the lower bowel may be of far greater functional significance than has been appreciated heretofore.

Methods

SUBJECTS

The subjects for the study were 12 of 14 male patients who were admitted for diagnostic colonoscopy. From their history it was considered that these patients were unlikely to have gross colonic pathology and they were invited to

participate. They agreed that the study could be carried out if, at colonoscopy, the colon was found to be unremarkable. The purpose of the study was explained and each gave their permission for the study in the full recognition that they could withdraw at any time without prejudice. The Southampton Hospitals' Ethical Committee gave approval for the conduct of the study.

Two patients, with a history of a change in bowel habit, were excluded because they had macroscopic colitis at colonoscopy which required mucosal biopsy. This left a total of 12 subjects who had a normal colon. Bowel preparation included a low residue diet for two days before the examination; on the day before colonoscopy sennosides 1 mg/kg (X-Prep, Napp Laboratories) and picosulphate, 10 mg (Picolax, Ferring pharmaceuticals). On the morning of the examination picosulphate, 10 mg, was taken and oral intake was then restricted to clear fluids. It was possible to see the whole colon in all cases.

A sample of urine was collected from all subjects before the examination for the measurement of baseline enrichment in urea. A measured dose of ^{30}N -urea (97.7 atom % ^{15}N , MSD Isotopes, Canada) was dissolved in normal saline. At the completion of the colonoscopy 1.5 mg/kg of ^{30}N -urea was injected through the biopsy channel of the colonoscope; into the caecum in six and distal to the splenic flexure in six. The isotope was flushed through with 10 ml normal saline and the colonoscope was removed without aspirating from the lumen.

All urine and stools passed in the next 72 hours were collected and stored in 6 mol HCl at -20°C . Urine was collected in four aliquots for 12, 12, 24, and 24 hours where possible. Stool was passed in day two by eight subjects and in day three by four. The stools were weighed, homogenised in water and a sample saved for later analysis. Two subjects who passed stool on day two were unable to save the specimen.

The concentration of urea and ammonia nitrogen in urine was measured using the Berthelot method.¹⁴ Urea nitrogen was isolated from the urine for mass spectrometry using a short ion exchange column.¹⁵ After digestion of the stool, the nitrogen content was measured in an automatic analyser (Kjeltec Auto 1030, Tecator Sweden). A further sample of stool was digested, to convert the nitrogen to ammonium sulphate for mass spectrometry.¹⁵ The nitrogen from urea and ammonium sulphate was liberated by reaction with lithium hypobromite. In this reaction the two nitrogen atoms from a single molecule of urea go to form a single molecule of nitrogen gas in a monomolecular reaction.¹⁶ Thereby it is possible to differentiate a molecule of nitrogen gas derived from ^{30}N -urea, ^{29}N -urea or ^{28}N -urea. Enrichment was measured in a triple collector isotope ratio mass spectrometer (SIRA 10, VG Isogas, Winsford, Cheshire).

Figure 1: Diagrammatic representation of the movement of urea nitrogen in the body. Urea nitrogen is formed from amino acids in the liver, and on an intake of 14 g N about 9.7 g urea is produced each day, 8.5 g from amino acids and 1.2 g coming from hydrolysed urea in the bowel.¹¹ Only 6.7 g of the urea produced is excreted in the urine with the remainder passing to the colon, 0.35 g through the ileocaecal valve and 2.65 g through the colonic wall. The urea is hydrolysed and of the 3.0 g nitrogen, 0.4 g is excreted in the stool, 1.2 g returning to urea synthesis and 1.4 g going to the synthesis of amino acids.

Results

The 12 patients completed the study. Most of the label was excreted in the first 12 to 24 hours. Peak enrichment occurred within 24 hours and declined towards baseline in the 72 hour period. A typical urinary enrichment pattern is outlined

Figure 2: Pattern of excretion of label in urine after the colonic instillation of labelled urea. In the top panel the relative increase in enrichment in the ratio of 30:28 urea (●), and 29:28 urea (○) with time shows an early peak falling to background levels of enrichment by 72 hours. The lower panel shows the amount of label excreted in the form of either 30N-urea (●) or 29N-urea (○) with maximal excretion during the first 24 hours, falling to zero by 72 hours.

in Figure 2 together with the amount of label excreted in milligrams over time. The label was excreted as both 30N-urea and 29N-urea in all subjects, although there was variation between individuals. The percentage of the dose excreted as urinary 30N-urea in the 12 subjects is shown in Figure 3. A similar proportion of the dose was recovered from the right colon, 6%, as was recovered from the left colon, 4%.

The Table shows the percentage of the dose

Figure 3: Urinary recovery of 30N-urea. Isotopically labelled 30N-urea was instilled into the colon and the excretion followed in urine for the next 72 hours. Similar proportions of the dose of label were recovered from urine in the following 72 hours when the isotope was placed in either the right or left colon.

Figure 4: Fate of 30N-urea. Isotopically labelled 30N-urea was instilled into the colon and the label recovered in stool or urine over the next 72 hours as either 29N-urea or 30N-urea. A large proportion of the dose could not be accounted for in the excretions, having been retained by the body.

that was recovered in the urine either as 30N-urea or as 29N-urea in subjects in whom the label had been placed in either the right or left colon. Although a slightly greater proportion was recovered as 29N-urea from the right colon, 18%, compared with the left colon, 13%, the difference did not reach statistical significance. In the 10 subjects in whom stool was available for analysis, the recovery of the label in the stool was very low. In one subject 12% of the dose appeared in the stool, but for the others it was less than 4%, implying absorption of over 90% of the dose. Overall, on the basis of label that could not be accounted for in stool or urine, the retention of label was about 74% from the right colon and 82% from the left colon. Figure 4 shows the relative fate of the labelled urea between the right and the left colon. Given the range of interindividual variation, there was no significant difference on a group basis between the right and left sides.

Discussion

This study can provide, in part, the answers to three questions: is the colon permeable to urea, is urea hydrolysed in the colon, what is the fate of nitrogen from urea hydrolysed in the colon? The

TABLE Patients selected for study had a history of change of bowel habit/abdominal pain (1) or iron deficiency anaemia (2) or a check colonoscopy after a previous polypectomy (3). The recovery and retention of isotope are expressed as a percentage of the dose of 15N15N-urea placed in the right or left colon

n	Age (yrs)	History	Urinary 15N15N-urea	Urinary 15N14N-urea	Stool	Retention
Right colon						
1	21	2	5.8	6.0	0.4	88
2	55	3	9.1	29.0	12.0	52
3	72	1	6.8	28.1	1.4	64
4	56	3	4.9	8.5	3.2	83
5	46	1	9.0	28.0	—	63
6	77	1	1.8	5.2	—	93
Mean	54.5		6.2	17.5	4.25	74
Left colon						
7	56	2	4.2	21.3	0.7	85
8	66	1	9.6	3.0	0.4	87
9	53	3	1.8	5.3	3.0	90
10	46	1	2.7	12.1	0.7	85
11	45	3	5.3	34.0	3.1	57
12	55	2	1.0	1.9	1.0	96
Mean	53.5		4.1	12.9	1.48	82

use of the colonoscope to gain access to different parts of the colon offers a relatively simple approach that is readily acceptable to subjects who are having the procedure as part of their diagnostic investigations. One disadvantage is that it requires a vigorous bowel cleansing to allow visualisation and it is unclear what effect this is likely to have on normal physiological function. Using the biopsy channel as a route of access we placed known quantities of labelled urea in either the caecum or just distal to the splenic flexure. We chose to study the two sites as it has been suggested that different parts of the colon may be functionally distinct.¹⁷ Furthermore it has been claimed that material placed in the caecum might reflux into the ileum,¹⁸ thereby giving misleading information. We have not been able to show any major difference between the two sites.

The dose of 15N15N-urea administered is about 30% of the endogenous urea hydrolysed in one hour.

The recovery of a small but significant proportion of the dose of urea as 30N-urea in urine shows that the colon is permeable to the urea molecule. In preliminary studies we were able to show 30N-urea in very early samples of urine, which suggests that available urea passes across the mucosa rapidly. These conclusions contrast with those reached by earlier workers, based upon studies in which the colon was perfused.^{2,3} When one considers that the normal transit time is 60 to 72 hours, 40 to 60 hours of which is spent in the colon,¹⁹ it is probable that perfusion studies do not always represent normal function.

Very little of the label was recovered in stool. This finding is in keeping with other studies which have shown that urea is normally absent from the stool and that after the administration of labelled urea, any label appearing in the stool is present in protein rather than in the native urea molecule.⁸ It is accepted that urea hydrolysis is predominantly a function of the colonic microflora.⁵ As urea is not known to participate in further metabolic interaction without hydrolysis, it can be assumed that the vast majority of the dose of label, greater than 90%, was hydrolysed in the colonic lumen. Of this about 4% was incorporated into faecal nitrogen, with the remainder being available for metabolic interaction in the host.

Studies in both animal models and in man have shown that if ammonia labelled with 15N is present in the portal vein, then the majority of the label will be directly incorporated into urea synthesis in the liver.²⁰ Therefore, the finding that, of the available label, about 15 to 20%, appeared as urinary 29N-urea, suggests that only one fifth of the urea nitrogen was being absorbed as ammonia with the remainder crossing the colonic wall in some other form. Heine *et al.*²¹ have recently shown that, in children with a colostomy, when a dose of 15N yeast protein was instilled into the colon, about 90% of the dose of 15N was absorbed and retained. The implication of these observations is that the colonic wall is permeable to the intact amino acids, derived from the yeast proteins, after digestion by the colonic microflora and that these amino acids are absorbed in significant quantities.

One possible interpretation of our data is that the microflora can utilise the urea instilled into the colon to satisfy their nitrogen requirements for amino acid synthesis; these amino acids then being available to the host. If a large proportion was retained within the bacterial cell then a far greater amount would have been recovered in the stool. This interpretation is in keeping with our earlier suggestion that the metabolic activity of the colonic flora may play an important role in the provision of essential and non-essential amino acids to the host, and that this function represents an important point of interaction between dietary fibre and protein requirements.¹²

Colonoscopy requires a bowel preparation, therefore the colon does not have its normal bacterial flora. Our findings, however, taken in conjunction with those of Heine *et al.*²¹ suggest that the absorptive capacity of the human colon has been underestimated by perfusion studies. It is likely that the intestinal flora affects the colonic handling of urea and the results may be markedly different in the presence of a normal mass of colonic microflora.

We acknowledge the continuing support of Mr S J Karran. We thank Dr Bamforth and Mr Royle for allowing us to study patients under their care, and our colleagues who performed the endoscopies. We are grateful for the support of The Rank Prize Fund; The Rank Foundation; B Braun; Hedley Foundation; and the Wessex Medical School Trust.

- 1 Waterlow JC. Observations on the mechanism of adaptation to low protein intakes. *Lancet* 1968; ii: 1091-7.
- 2 Wolpert E, Phillips SF, Summerskill WHJ. Transport of urea and ammonia production in the human colon. *Lancet* 1971; ii: 1387-90.
- 3 Bown RL, Gibson JA, Fenton JCB, Snedden W, Clark ML, Sladen GE. Ammonia and urea transport by the excluded human colon. *Clin Sci Mol Med* 1975; 48: 279-87.
- 4 Walser M, Bodenlos LJ. Urea metabolism in man. *J Clin Invest* 1959; 38: 1617-26.
- 5 Richards P. Nutritional potential of nitrogen recycling in man. *Am J Clin Nutr* 1972; 25: 615-25.
- 6 Chadwick VS, Jones JD, Debonnie JC, Gaginella T, Phillips SF. Urea, uric acid and creatinine fluxes through the small intestine in man. [Abstract]. *Gut* 1977; 18: A944.
- 7 Gibson JA, Park NJ, Sladen GE, Dawson AM. The role of the colon in urea metabolism in man. *Clin Sci Mol Med* 1976; 50: 51-9.
- 8 Wrong OM, Vince AJ, Waterlow JC. The contribution of endogenous urea to faecal ammonia in man, determined by 15N labelling of plasma urea. *Clin Sci* 1985; 68: 193-9.
- 9 Schafer DF. In hepatic coma the problem comes from the colon, but will the answers come from there. *J Lab Clin Med* 1987; 110: 253-4.
- 10 Jackson AA. Dynamics of protein metabolism and their relation to adaptation. In: Taylor TG, Jenkins NK, eds. *Proc of the XIII International Congress of Nutrition* 1985. London: John Libbey; 1986: 403-9.
- 11 Jackson AA, Picou D, Landman J. The non-invasive measurement of urea kinetics in normal man by a constant infusion of 15N15N-urea. *Hum Nutr Clin Nutr* 1984; 38: 339-54.
- 12 Jackson AA. Amino acids: essential and non-essential. *Lancet* 1983; i: 1034-7.
- 13 Houtp TR, Houtp KA. Transfer of urea nitrogen across the rumen wall. *Am J Physiol* 1968; 214: 1296-303.
- 14 Kaplan A. Urea nitrogen and ammonia nitrogen. In: Meites S, ed. *Standard methods of clinical chemistry* 1965; 5: 245-56.
- 15 Jackson AA, Golden MHN, Jahoor PF, Landman J. The isolation of urea nitrogen and ammonia nitrogen from biological samples for mass spectrometry. *Anal Biochem* 1980; 105: 14-7.
- 16 Walser M, George J, Bodenlos LJ. Altered proportions of isotopes of molecular nitrogen as evidence for a monomolecular reaction. *J Chem Phys* 1959; 22: 1146.
- 17 Stevens CE. Comparative physiology of the vertebrate digestive system. In: Blaxter K, MacDonald I, eds. *Comparative nutrition*. London: John Libbey; 1988: 21-36.
- 18 Devroede GJ, Phillips SF. Studies of the perfusion technique for colonic absorption. *Gastroenterology* 1969; 56: 92-100.
- 19 Cummings JH. Microbial digestion of complex carbohydrates in man. *Proc Nutr Soc* 1984; 43: 35-44.
- 20 Nissim I, Yudkoff M, Yang W, Terwilliger T, Segal S. Rapid gas chromatography-mass spectrometric analysis of [15N]urea: application to human metabolic studies. *Clin Chim Acta* 1981; 109: 295-304.
- 21 Heine W, Wutzke KD, Richter I, Walther P, Plath C. Evidence for colonic absorption of protein nitrogen in infants. *Acta Paediatr Scand* 1987; 76: 741-4.