Uremic solutes from colon microbes

Timothy W. Meyer[1] and Thomas H. Hostetter[2]

[1]Department of Medicine, VA Palo Alto HCS and Stanford University, Palo Alto, California, USA and [2]Case Western University School of Medicine, Cleveland, Ohio, USA

There is renewed interest in identifying organic waste solutes that are normally excreted by the kidneys and must be removed by renal replacement therapy when the kidneys fail. A large number of these waste solutes are produced by colon microbes. Mass spectrometry is expanding our knowledge of their chemical identity, and DNA sequencing technologies are providing new knowledge of the microbes and metabolic pathways by which they are made. There is evidence that the most extensively studied of the colon-derived solutes, indoxyl sulfate and p-cresol sulfate, are toxic. Much more study is required to establish the toxicity of other solutes in this class. Because they are made in an isolated compartment by microbes, their production may prove simpler to suppress than the production of other waste solutes. To the extent that they are toxic, suppressing their production could improve the health of renal failure patients without the need for more intensive or prolonged dialysis.

Kidney International (2012) **81,** 949–954; doi:10.1038/ki.2011.504; published online 8 February 2012

KEYWORDS: hemodialysis; nutrition; uremic toxins

Correspondence: *Timothy W. Meyer, Department of Medicine, VA Palo Alto HCS and Stanford University, Nephrology 111R, 3801 Miranda Avenue, Palo Alto, California 94303, USA. E-mail: twmeyer@stanford.edu*

Received 9 September 2011; revised 13 October 2011; accepted 18 October 2011; published online 8 February 2012

The kidney excretes a large number of organic waste solutes whose origins are diverse. Better knowledge of these solutes could lead to improved treatment for patients with kidney failure. Many waste solutes are produced by colon microbes. There is reason to suppose that some of these compounds, which are foreign to mammalian metabolism, have toxic effects. Because they are made in an isolated body compartment by microbes, their production may prove simpler to suppress than the production of other uremic waste solutes.

THE COLON MICROBIOME

Our human bodies are heavily colonized by microbes.[1] The largest collection of these microbes, with a density of 10^{10} to 10^{12} cell/ml, inhabits the colon.[1–3] In cell number and even more in gene number, the colon microbial population or colon 'microbiome' dwarfs its human host. DNA sequencing technology has greatly increased our knowledge of the diversity and function of the colon microbes. In mammalian evolution, their chief value has been to extract energy from plant polysaccharides that cannot be digested by host enzymes. Microbial fermentation of these polysaccharides yields short chain fatty acids. While this process provides only a small portion of the energy used daily in humans consuming an 'industrialized' diet, it can be a crucial source of energy when the diet contains more unprocessed plant food.[4] Colon microbes also provide micronutrients including vitamins and produce hormones that promote fat storage. Through co-evolution with their mammalian hosts, they have developed the capacity to limit colonization of the gut by pathogens and to stimulate the host immune system in useful ways. The action of colon microbes on foodstuffs and intestinal secretions, however, generates numerous organic compounds in addition to those that are useful to their host.[5] The burden of excreting some of these compounds in the urine represents a biological price paid by the host for harboring the microbes. Colon-derived solutes that are normally excreted in the urine accumulate in the plasma when the kidneys fail and may contribute to uremic toxicity.[6,7]

UREMIC SOLUTES PRODUCED BY COLON MICROBES

While knowledge of the colon's microbial diversity is new, the hypothesis that colon microbes produce toxic solutes is old. In his original description of hemodialysis, Kolff[8] noted that

"Besides indoxyl still other various putrefaction products of the intestine may be increased in the blood in renal insufficiency [sic]. They are phenoles, cresoles, aromatic oxyacids, and other aromatic substances."

Putrefaction—the breakdown of proteins by bacteria—excited both scientific and popular interest in the late nineteenth and early twentieth centuries.[9] The Russian Nobel Laureate Metchnikoff believed that in modern humans the colon was not only useless but harmful, and that 'autointoxication' caused by colonic putrefaction accelerated aging and caused disease. He advocated the consumption of milk soured by lactic acid–producing bacteria to inhibit proteolysis in the colon. The British surgeon W. Arbuthnot Lane went further and bypassed the colon to treat diseases ranging from tuberculosis to rheumatoid arthritis.[9] The autointoxication theory had been largely discredited by Kolff's time, but it had left behind a legacy of solid biochemical research. Gut bacteria had been shown to degrade tryptophan into a variety of indoles and phenylalanine and tyrosine into a variety of phenyl compounds. Some of these compounds were found first in the urine of normal humans and then in the plasma of patients with kidney failure.[10]

The colon-derived uremic solutes that have been most extensively investigated are indoxyl sulfate and p-cresol sulfate, which are formed as depicted in Figure 1.[11,12] These compounds have received the most attention because they are formed in relatively large quantities and therefore proved easiest to measure. As reviewed below, there is considerable, though not conclusive, evidence that they are toxic. Indoxyl sulfate and p-cresol sulfate are only two, however, of a very large number of colon-derived uremic solutes. Other compounds in this class may be more toxic, although they are present in lower concentration. Between 1940 and 1990, numerous indoles and phenols were identified in uremic plasma first by paper and then gas chromatography.[10,13,14] Researchers hypothesized that many of them were made by colon microbes. In most cases, however, their origin was not confirmed and there has been very little evaluation of their toxicity.

The application of mass spectrometry has the power to greatly enlarge our knowledge of uremic solutes in general and of colon-derived uremic solutes in particular.[5,15,16] High-resolution detectors that resolve the mass to charge (m/z) ratios of ions to within a few parts per million may prove particularly valuable in uremia research. These instruments make it possible to perform 'nontargeted' metabolomic studies in which numerous unnamed chemical features characterized only by their m/z ratios are detected along with known compounds. Kikuchi *et al.*[17] first used nontargeted mass spectrometry to detect solutes that accumulate in the plasma of rats with renal insufficiency, and Sato *et al.*[18] have recently demonstrated the capacity of nontargeted mass spectrometry to detect both known and unknown compounds in a study evaluating the effect of hemodialysis on plasma solute concentrations.

Solutes produced by colon microbes can be identified by combining mass spectrometry with maneuvers that suppress microbial solute production. A pioneering study by Wikoff *et al.*[5] used high-resolution mass spectrometry to identify plasma solutes produced by gut microbes in rats. Of several thousand features detected in plasma, ~10 percent was considered to vary in concentration between conventional and germ-free rats. The majority of these features was less prevalent in the germ-free animals, and thus considered to be of microbial origin. Of note, some features considered to be of microbial origin were later found to accumulate in the plasma of mice lacking the renal anion transporter OAT1, suggesting that they are largely removed from the body by renal tubular secretion.[19] Kikuchi *et al.*[20] subsequently used mass spectrometry to identify solutes in whose plasma levels

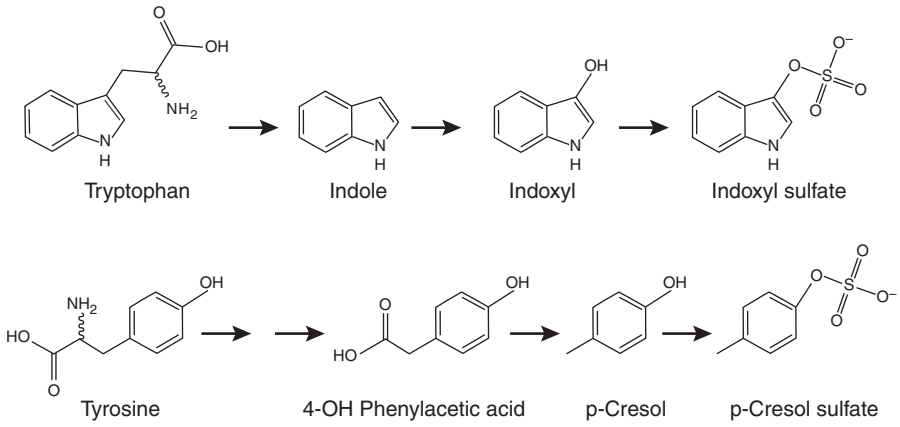

Figure 1 | Microbial generation of indoxyl sulfate from tryptophan and of p-cresol sulfate from tyrosine. Tryptophan is converted to indole by tryptophanase, which is found only in microbes. Absorption of indole in the colon is then followed by oxidation and sulfation in the liver. Tyrosine is converted by microbes in two steps to 4-hydroxy phenylacetic acid, which is then decarboxylated to p-cresol by an enzyme that has been shown to be present most notably in *Clostridium difficile*.[47] Sulfation of p-cresol is then accomplished in the colonic epithelium and possibly also in the liver. Older reports measured p-cresol rather than p-cresol sulfate in the plasma of dialysis patients because assay techniques used acidification that hydrolyzed the conjugate.[12] Lesser portions of both indoxyl and p-cresol are conjugated with glucuronic acid rather than sulfuric acid.

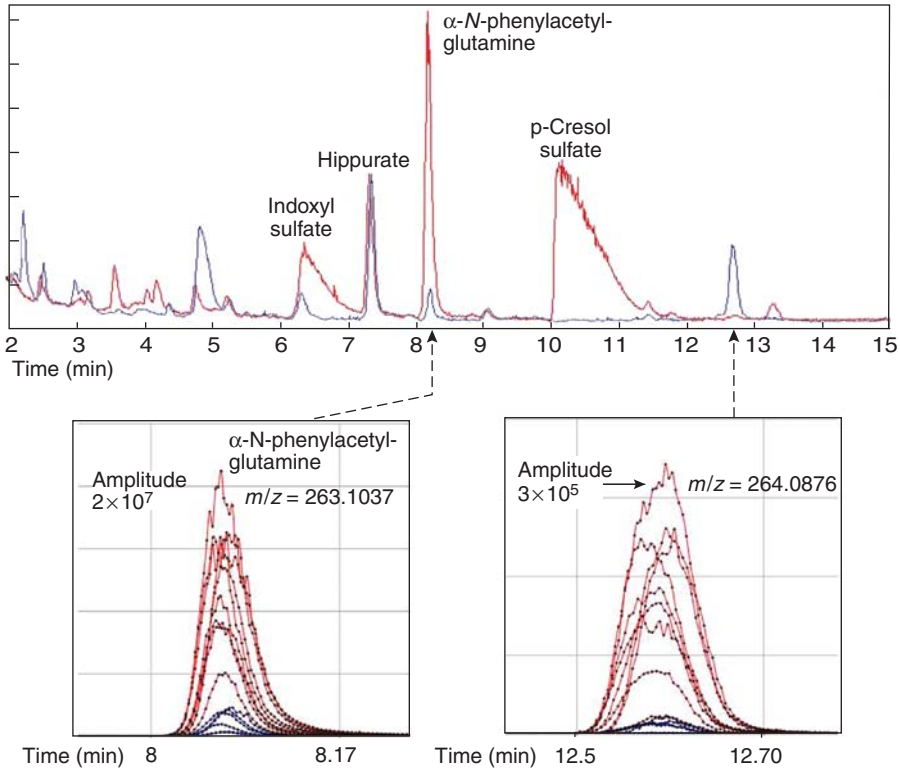

Figure 2 | The identification of colon-derived solutes by mass spectrometry. The upper part of the figure shows total ion current in the negative mode vs. time obtained using liquid chromatography/mass spectrometry to analyze plasma from a single patient with an intact colon (red line) as compared with a single patient who had a colectomy (blue line). The total ion current reflects the sum of all ions, regardless of their individual mass, entering the mass spectrometer at each time point. Peaks reflecting the presence of large amounts of indoxyl sulfate, α-*N*-phenylacetyl-glutamine, and p-cresol sulfate in the plasma from a dialysis patient with an intact colon are not seen in the patient who had a colectomy. The lower figures illustrate the power of nontargeted mass spectrometry to detect individual solutes identified by mass charge (m/z) ratio. They compare ion current at two m/z values obtained on analysis of plasma from nine hemodialysis patients with intact colons (red lines) as compared with six hemodialysis patients who had colectomies (blue lines). The figure on the left confirms that α-*N*-phenylacetyl-glutamine, *m/z* 263.1037, is present in lower concentration in patients who had colectomies. The identity of this peak as α-*N*-phenylacetyl-glutamine was confirmed by analysis of a chemical standard. The figure on the right illustrates that a solute with *m/z* 264.0876 is also present in much lower concentration in patients who had colectomies. No compound matching this m/z ratio is included in standard databases of human metabolites and its identity remains to be determined. Note that the signal for this feature is of much lower amplitude than that for α-*N*-phenylacetyl-glutamine and that it is found at a time point where no peak is visible on the total ion chromatogram from the patient with a colon, although an unrelated peak is present on the total ion chromatogram from the patient who had a colectomy. Nontargeted mass spectrometry reveals that the total ion chromatogram obtained from plasma samples is comprised of a very large number of individual features including many of relatively low magnitude. Data are from the report of Aronov *et al.*[21]

were reduced by the administration of the oral sorbent AST-120 in rats with renal insufficiency. We recently used an approach similar to that used by Wikoff *et al.*[5] to compare plasma solute profiles in dialysis patients with and without colons.[21] The difference between patients with and without colons was readily apparent on superficial examination of mass spectra, as illustrated in Figure 2. Statistical analysis identified more than 20 mass spectrometric features that were colon-derived. Of note, the great majority of these was more prominent in dialysis patients than normal subjects and so could be considered to represent uremic solutes.

A weakness of nontargeted mass spectrometry is that it provides mass values but not chemical identities for the numerous features detected. Chemical identification requires further analysis of samples containing reagent standards and/or 'MS/MS' measurements of the ion masses produced by fragmentation of the original ions.[22] Only a small number of the colon-derived solutes recently detected by mass spectrometry have been chemically identified. Wikoff *et al.*[5] identified only 16 of several hundred features that were more prominent in the plasma of conventional than germ-free rats. These included three indole and six phenyl compounds. We identified three indole and two phenyl compounds that were present at higher concentration in dialysis patients with colons than in dialysis patients without colons and normal subjects.[21] Part of the problem is that organic waste compounds are often conjugated to form sulfates, glucuronides, or other complex ions. Conjugation, as illustrated for p-cresol and indoxyl in Figure 1, can at once reduce the toxicity of waste compounds and facilitate their excretion by tubular secretion. But conjugation makes it harder to determine the chemical identity of features for which mass spectrometry provides only the m/z ratio. Standard databases usually do not contain mass values for conjugates of

Table 1 | Colon-derived uremic solutes

	References
Indole compounds	
Indoxyl sulfate	5, 20, and 21
Indoxyl glucuronide	21 and 45
5-Hydroxyindole	21
Indole-3-propionic acid	5
Phenyl compounds	
p-Cresol sulfate	5, 20, and 21
p-Cresol glucuronide	46
Phenyl sulfate	5 and 20
Phenyl glucuronide	21
α-*N*-phenylacetyl-L-glutamine	5 and 21
Phenylpropionylglycine	5
Cinnamoylglycine	5 and 21
4-Ethylphenyl sulfate	20
Hippuric acid	5 and 20

This list, based heavily on references 5, 19, and 20, is certainly incomplete. Indole-3-propionic acid and phenylpropionylglycine were identified by Wikoff *et al.*[5] as microbial products in rat plasma and other studies have demonstrated their excretion in the urine. The results of Wikoff *et al.*[5] suggest that hippuric acid is largely of microbial origin in rats, but those of Aronov *et al.*[21] suggest important nonmicrobial production of hippuric acid in humans.

microbial metabolites and reagent standards for such compounds are rarely available.

THE POTENTIAL TOXICITY OF COLON-DERIVED SOLUTES

Colon-derived uremic solutes that have been identified to date are listed in Table 1. The list will get longer as more mass spectrometric studies are performed. We will thus face an increasingly large task in determining the extent to which any of these solutes are toxic. Indoxyl sulfate has been shown to be toxic *in vitro*, and levels of p-cresol sulfate have been correlated with poor outcomes in dialysis patients.[6,7,23,24] Recent studies have concentrated on the possibility that these compounds cause vascular injury.[11,25,26] The structural relation of the uremic indoles and phenols to neurotransmitters previously encouraged speculation that they impair cognitive function, and it has recently been noted that they could do this by interfering with cleansing of the brain interstitium by the blood–brain barrier.[27] A separate body of work suggests that indoxyl sulfate injures renal tubular cells and may thereby contribute to the progression of renal insufficiency.[11] While indoxyl sulfate and p-cresol sulfate are the only colon-derived solutes that have been studied extensively to date, recent metabolomic studies suggest that other solutes derived from gut bacteria are also risk factors for cardiovascular disease in humans and appear to have causative roles as judged by animal studies.[28]

REDUCING THE LOAD OF COLON-DERIVED SOLUTES

Most efforts to lower uremic solute levels have focused on increasing solute removal and ignored the alternate strategy of suppressing solute production. Maneuvers to suppress production, however, could prove particularly effective for solutes that are made in an isolated body compartment by microbes. In addition, maneuvers that suppress the production of multiple colon-derived solutes could be tested without first establishing which of these solutes are most toxic.

One potential means to limit the production of colon-derived solutes is to alter the food supplied to colon microbes. The known colon-derived solutes are derived from amino acids, as is illustrated for indoxyl sulfate and p-cresol sulfate in Figure 1. On an 'industrialized' diet, the colon microbes are supplied with ~10 g of amino acids in the form of incompletely digested proteins, sloughed intestinal cells, and secretions.[4,29] There is evidence that intestinal protein absorption is impaired in renal failure, so that the portion of ingested protein delivered to the colon microbes is increased.[30] One means to limit this supply is to restrict protein intake. Before dialysis became available, uremic symptoms were relieved by dietary protein restriction and reduced production of colon-derived solutes may have contributed to the effectiveness of this treatment. Protein restriction, however, is unpalatable and can cause negative nitrogen balance. Increasing dietary fiber intake could provide a simpler and safer means to reduce the production of colon-derived uremic solutes.[31] The term fiber includes both nonstarch polysaccharides and resistant starches that escape digestion in the small intestine.[31,32] Industrialized diets contain less fiber than primitive diets, and dialysis patients consume even less fiber than their healthy neighbors.[33] With low-fiber intake the supply of carbohydrates to the colon microbes is low.[34] With limited substrate for fermentation, microbial growth is reduced and the volume of the stool, of which microbes make up a major portion, is reduced. The amount of amino acids needed for synthesis of microbial protein is thus reduced, so that an increased portion of the proteins and peptides delivered to the colon is converted into uremic solutes. A prolonged transit time through the colon, which results in part from lesser microbial growth, promotes conversion of amino acids to uremic wastes.[35] Increasing dietary fiber intake is predicted to have the opposite effects and to limit the conversion of amino acids to uremic solutes while increasing microbial growth. Higher fiber intake could account for the observation that urinary excretion of indoxyl sulfate and p-cresol sulfate is lower in vegetarians than in people eating an unrestricted diet. In dialysis patients, supplementing the diet with 10–20 g/day of non-digestible carbohydrates was recently shown to lower plasma levels of p-cresol sulfate, which should encourage further efforts in this direction.[31] An interesting, but as yet unexplained, finding is that production of the colon-derived solutes indoxyl sulfate and p-cresol sulfate appears to be lower in patients on peritoneal dialysis than in patients on hemodialysis.[36]

Another potential means to reduce the daily load of colon-derived solutes is to administer sorbents that bind to microbial metabolites. The carbon-based sorbent AST-120 reduces plasma levels of indoxyl sulfate and can also reduce levels of p-cresol sulfate and other solutes.[20] Clinical interest in AST-120 is currently focused on testing whether it can slow the progression of renal insufficiency. However, sorbents could also be used to lower solute levels and improve health

in dialysis patients. Other maneuvers that could reduce the production of colon-derived solutes include treatment with laxatives to reduce transit time and antibiotics or 'probiotic' bacterial strains to alter the microbial population.[6,32] A particularly exciting possibility is that new knowledge of the colon microbiome will permit redirection of solute production by genetic manipulation of colon microbes.[37] An attractive feature of all these potential treatments is that they could lower solute levels without the burden of increasing dialysis duration and/or frequency.

LIVER AND KIDNEY

The liver and kidney together provide the body with an organic waste disposal system. Remarkably similar symptoms, including prominent encephalopathy, are caused by failure of either organ. Efforts to control uremic symptoms by reducing solute production largely ended with the development of dialysis. Hepatic encephalopathy, however, is still treated by maneuvers designed to suppress waste solute production. These maneuvers, moreover, are directed exclusively toward solutes made by colon microbes. Their initial target was ammonia, but other colon-derived solutes are thought also to contribute to hepatic encephalopathy, and the severity of symptoms is not well correlated with the plasma level of any known solute. The maneuvers that have been used to reduce solute production in liver failure include colectomy, restriction of dietary protein, and administration of non-digestible carbohydrates, laxatives, sorbents, antibiotics, and probiotics. These are the same maneuvers that could be used to reduce colon-derived solute production in renal failure, as described above. For many years, these maneuvers were used in the liver failure without proof of their efficacy. But controlled trials have recently established that the nonabsorbed disaccharide lactulose and the antibiotic rifaxamin maintain remission from hepatic encephalopathy.[38] Trials of maneuvers to suppress colon-derived solute production in renal failure may have to be larger and/or longer, given that symptoms of solute retention in dialysis patients are less severe. However, the successful trials in the liver failure show that chronic suppression of colon-derived solute production is feasible and can be beneficial.

INCREASING THE REMOVAL OF COLON-DERIVED SOLUTES

The alternative to suppressing solute production is to improve solute removal by dialysis. How easily this can be accomplished depends on the nature of the solute and its distribution in the body.[39] Indoxyl sulfate and p-cresol sulfate, the two best known colon-derived solutes, both bind to plasma albumin. This binding is avid but reversible. More than 90% of the solute in the plasma is bound to albumin at any instant, but the bound portion is in rapid equilibrium with the free portion. Because only free solutes are available for diffusion through the dialysis membrane, the clearances for indoxyl sulfate and p-cresol sulfate are only a small fraction of the urea clearance during conventional dialysis. However, the clearance of the bound solutes can be greatly increased by increasing the dialysate flow and size of the dialyzer.[40,41] The clearance of bound solutes can also be increased by combining high-volume ultrafiltration with hemodialysis and by use of sorbents.[42,43] Application of these techniques could reduce the time-averaged concentrations of bound solutes without increasing either the duration or frequency of dialysis. The extent of protein binding has not been evaluated for the majority of colon-derived solutes, but some of those listed in Table 1, such as p-cresol glucuronide and α-*N*-phenylacetyl-glutamine, are known not to be protein bound. For these solutes, suppression of production could provide a simpler means to lower solute levels than increasing dialytic removal. Recent studies have documented wide variability in the plasma levels of uremic solute levels among individual patients with the same degree of renal insufficiency, suggesting that solute production is variable and should be susceptible to manipulation.[44]

CONCLUSION

Colon microbes produce a large number of organic compounds that are foreign to mammalian cell metabolism. Some of these compounds are absorbed in the colon and then normally excreted from the body by the kidneys. The possibility that accumulation of these compounds contributes to uremic toxicity is strong enough to warrant trials of the clinical effect of lowering their levels in the plasma.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

Support to this study was provided by the National Institutes of Health (R21DK84439 and RO1 DK80123 to TWM and RO1 DK80123 to THH).

REFERENCES

1. Dethlefsen L, McFall-Ngai M, Relman DA. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature* 2007; **449**: 811–818.
2. Goodman AL, Gordon JI. Our unindicted coconspirators: human metabolism from a microbial perspective. *Cell Metab* 2010; **12**: 111–116.
3. Walter J, Britton RA, Roos S. Host-microbial symbiosis in the vertebrate gastrointestinal tract and the Lactobacillus reuteri paradigm. *Proc Natl Acad Sci USA* 2011; **108**(Suppl 1): 4645–4652.
4. Nordgaard I, Mortensen PB. Digestive processes in the human colon. *Nutrition* 1995; **11**: 37–45.
5. Wikoff WR, Anfora AT, Liu J *et al.* Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci USA* 2009; **106**: 3698–3703.
6. Evenepoel P, Meijers BK, Bammens BR *et al.* Uremic toxins originating from colonic microbial metabolism. *Kidney Int Suppl* 2009; **76**(Suppl 114): S12–S19.
7. Schepers E, Glorieux G, Vanholder R. The gut: the forgotten organ in uremia? *Blood Purif* 2010; **29**: 130–136.
8. Kolff WJ. *The Artificial Kidney*. J. H. Kok: Kampen, 1946.
9. Chen TS, Chen PS. Intestinal autointoxication: a medical leitmotif. *J Clin Gastroenterol* 1989; **11**: 434–441.
10. Ludwig GD, Senesky D, Bluemle Jr LW *et al.* Indoles in ureamia: identification by countercurrent distribution and paper chromatography. *Am J Clin Nutr* 1968; **21**: 436–450.
11. Niwa T. Indoxyl sulfate is a nephro-vascular toxin. *J Ren Nutr* 2010; **20**: S2–S6.
12. Vanholder R, Bammens B, de Loor H *et al.* Warning: the unfortunate end of p-cresol as a uraemic toxin. *Nephrol Dial Transplant* 2011; **26**: 1464–1467.

13. Niwa T, Maeda K, Ohki T *et al.* A gas chromatographic-mass spectrometric analysis for phenols in uremic serum. *Clin Chim Acta* 1981; **110**: 51–57.
14. Van Haard P, Pavel S. Chromatography of urinary indole derivatives. *J Chromatogr* 1988; **429**: 59–94.
15. Niwa T. Recent progress in the analysis of uremic toxins by mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009; **877**: 2600–2606.
16. Rhee EP, Souza A, Farrell L *et al.* Metabolite profiling identifies markers of uremia. *J Am Soc Nephrol* 2010; **21**: 1041–1051.
17. Kikuchi K, Itoh Y, Tateoka R *et al.* Metabolomic analysis of uremic toxins by liquid chromatography/electrospray ionization-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010; **878**: 1662–1668.
18. Sato E, Kohno M, Yamamoto M *et al.* Metabolomic analysis of human plasma from haemodialysis patients. *Eur J Clin Invest* 2011; **41**: 241–255.
19. Wikoff WR, Nagle MA, Kouznetsova VL *et al.* Untargeted metabolomics identifies enterobiome metabolites and putative uremic toxins as substrates of organic anion transporter 1 (Oat1). *J Proteome Res* 2011; **10**: 2842–2851.
20. Kikuchi K, Itoh Y, Tateoka R *et al.* Metabolomic search for uremic toxins as indicators of the effect of an oral sorbent AST-120 by liquid chromatography/tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010; **878**: 2997–3002.
21. Aronov P, Luo FY, Plummer NS *et al.* Colonic contribution to uremic solutes. *J Am Soc Nephrol* 2011; **22**: 1769–1776.
22. Roux A, Lison D, Junot C *et al.* Applications of liquid chromatography coupled to mass spectrometry-based metabolomics in clinical chemistry and toxicology: a review. *Clin Biochem* 2011; **44**: 119–135.
23. De Smet R, Van Kaer J, Van Vlem B *et al.* Toxicity of free p-cresol: a prospective and cross-sectional analysis. *Clin Chem* 2003; **49**: 470–478.
24. Bammens B, Evenepoel P, Keuleers H *et al.* Free serum concentrations of the protein-bound retention solute p-cresol predict mortality in hemodialysis patients. *Kidney Int* 2006; **69**: 1081–1087.
25. Faure V, Dou L, Sabatier F *et al.* Elevation of circulating endothelial microparticles in patients with chronic renal failure. *J Thromb Haemost* 2006; **4**: 566–573.
26. Meijers BK, Van Kerckhoven S, Verbeke K *et al.* The uremic retention solute p-cresyl sulfate and markers of endothelial damage. *Am J Kidney Dis* 2009; **54**: 891–901.
27. Hosoya KI, Tachikawa M. Roles of organic anion/cation transporters at the blood-brain and blood-cerebrospinal fluid barriers involving uremic toxins. *Clin Exp Nephrol* 2011; **15**: 478–485.
28. Wang Z, Klipfell E, Bennett BJ *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011; **472**: 57–63.
29. Chacko A, Cummings JH. Nitrogen losses from the human small bowel: obligatory losses and the effect of physical form of food. *Gut* 1988; **29**: 809–815.
30. Bammens B, Verbeke K, Vanrenterghem Y *et al.* Evidence for impaired assimilation of protein in chronic renal failure. *Kidney Int* 2003; **64**: 2196–2203.
31. Meijers BK, De Preter V, Verbeke K *et al.* p-Cresyl sulfate serum concentrations in haemodialysis patients are reduced by the prebiotic oligofructose-enriched inulin. *Nephrol Dial Transplant* 2009; **25**: 219–224.
32. De Preter V, Hamer HM, Windey K *et al.* The impact of pre- and/or probiotics on human colonic metabolism: does it affect human health? *Mol Nutr Food Res* 2011; **55**: 46–57.
33. Kalantar-Zadeh K, Kopple JD, Deepak S *et al.* Food intake characteristics of hemodialysis patients as obtained by food frequency questionnaire. *J Ren Nutr* 2002; **12**: 17–31.
34. Elia M, Cummings JH. Physiological aspects of energy metabolism and gastrointestinal effects of carbohydrates. *Eur J Clin Nutr* 2007; **61**(Suppl 1): S40–S74.
35. Stephen AM, Wiggins HS, Cummings JH. Effect of changing transit time on colonic microbial metabolism in man. *Gut* 1987; **28**: 601–609.
36. Pham NM, Recht NS, Hostetter TH *et al.* Removal of the protein-bound solutes indican and p-cresol sulfate by peritoneal dialysis. *Clin J Am Soc Nephrol* 2008; **3**: 85–90.
37. Goodman AL, Kallstrom G, Faith JJ *et al.* Extensive personal human gut microbiota culture collections characterized and manipulated in gnotobiotic mice. *Proc Natl Acad Sci USA* 2011; **108**: 6252–6257.
38. Riordan SM, Williams R. Gut flora and hepatic encephalopathy in patients with cirrhosis. *N Engl J Med* 2010; **362**: 1140–1142.
39. Meyer TW, Sirich TL, Hostetter TH. Dialysis cannot be dosed. *Semin Dial* 2011; **24**: 471–479.
40. Meyer TW, Leeper EC, Bartlett DW *et al.* Increasing dialysate flow and dialyzer mass transfer area coefficient to increase the clearance of protein-bound solutes. *J Am Soc Nephrol* 2004; **15**: 1927–1935.
41. Luo FJ, Patel KP, Marquez IO *et al.* Effect of increasing dialyzer mass transfer area coefficient and dialysate flow on clearance of protein-bound solutes: a pilot crossover trial. *Am J Kidney Dis* 2009; **53**: 1042–1049.
42. Meijers BK, Bammens B, Verbeke K *et al.* A review of albumin binding in CKD. *Am J Kidney Dis* 2008; **51**: 839–850.
43. Meert N, Waterloos MA, Van Landschoot M *et al.* Prospective evaluation of the change of predialysis protein-bound uremic solute concentration with postdilution online hemodiafiltration. *Artif Organs* 2010; **34**: 580–585.
44. Eloot S, Schepers E, Barreto DV *et al.* Estimated glomerular filtration rate is a poor predictor of concentration for a broad range of uremic toxins. *Clin J Am Soc Nephrol* 2011; **6**: 1266–1273.
45. Niwa T, Miyazaki T, Tsukushi S *et al.* Accumulation of indoxyl-beta-D-glucuronide in uremic serum: suppression of its production by oral sorbent and efficient removal by hemodialysis. *Nephron* 1996; **74**: 72–78.
46. Lesaffer G, De Smet R, Belpaire FM *et al.* Urinary excretion of the uraemic toxin p-cresol in the rat: contribution of glucuronidation to its metabolization. *Nephrol Dial Transplant* 2003; **18**: 1299–1306.
47. Dawson LF, Donahue EH, Cartman ST *et al.* The analysis of para-cresol production and tolerance in Clostridium difficile 027 and 012 strains. *BMC Microbiol* 2011; **11**: 86.