Review Article

Clinical Applications of Urinary Organic Acids. Part 2. Dysbiosis Markers

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Abstract

Part 1 of this series focused on urinary organic acids as markers of detoxification; part 2 focuses on dysbiosis markers. Intestinal microbial growth is accompanied by the release of products of their metabolism that may be absorbed and excreted in urine. Several organic acids are known to be specific products of bacterial metabolic action on dietary polyphenols or unassimilated amino acids or carbohydrates. Associated gastrointestinal or neurological symptoms may result from irritation of the intestinal mucosa or systemic distribution of absorbed neurotoxic products. Detection of abnormally elevated levels of these products is a useful diagnostic tool for patients with gastrointestinal or toxicological symptoms. Test profiles of urinary organic acids associated with microbial overgrowth can include benzoate, hippurate, phenylacetate, phenylpropionate, cresol, hydroxybenzoate, hydroxyphenylacetate, hydroxyphenylpropionate and 3,4-dihydroxyphenylpropionate, indican, tricarballylate, D-lactate, and D-arabinitol. Effective treatments for the associated microbial overgrowths may be directed at reducing microbial populations, introducing favorable microbes, and restoring intestinal mucosal integrity. (*Altern Med Rev* 2008;13(4):292-306)

Introduction

By acting on various dietary or endogenous substrates, intestinal bacteria or parasites can generate metabolic products that are absorbed and excreted in urine with or without further modification in the liver and kidney. Dietary polyphenols have been shown to be one of the dominant substrates for yielding phenolic compounds, whereas dietary simple sugars lead to generation of non-phenolic hydrocarbon products. Although there are numerous polyphenolic chemical structures contained in foods, it appears that a relatively small number of phenolic products are formed.[1] This means that variations in specific food consumption from one patient to the next may have only small effects on the potential for generating phenolic products. The greater factor in the production of phenolic compounds in the gut is the type and activity of the microbes that are present.[2]

The anatomical region of the gut most likely to yield bacterial metabolites is the middle or transitional gut, including the terminal ileum and the ascending colon. This most difficult region to assess by direct examination is the primary origin of urinary microbial products because the passing of chyme to the lower ileum corresponds to the lag phase for the onset of logarithmic growth rates characteristic of most bacteria.[3] It is during this most intense growth phase, when the microbial counts rise from 10^5 to 10^{11}/g, that metabolic products are most actively produced. Thus, by measuring their products in urine, information principally about the mid- or transitional-gut microbial mass is obtained. Microbes that do not effectively compete with the dominant species in the colon may produce elevated urinary markers, even when they are not detected in stool specimens.

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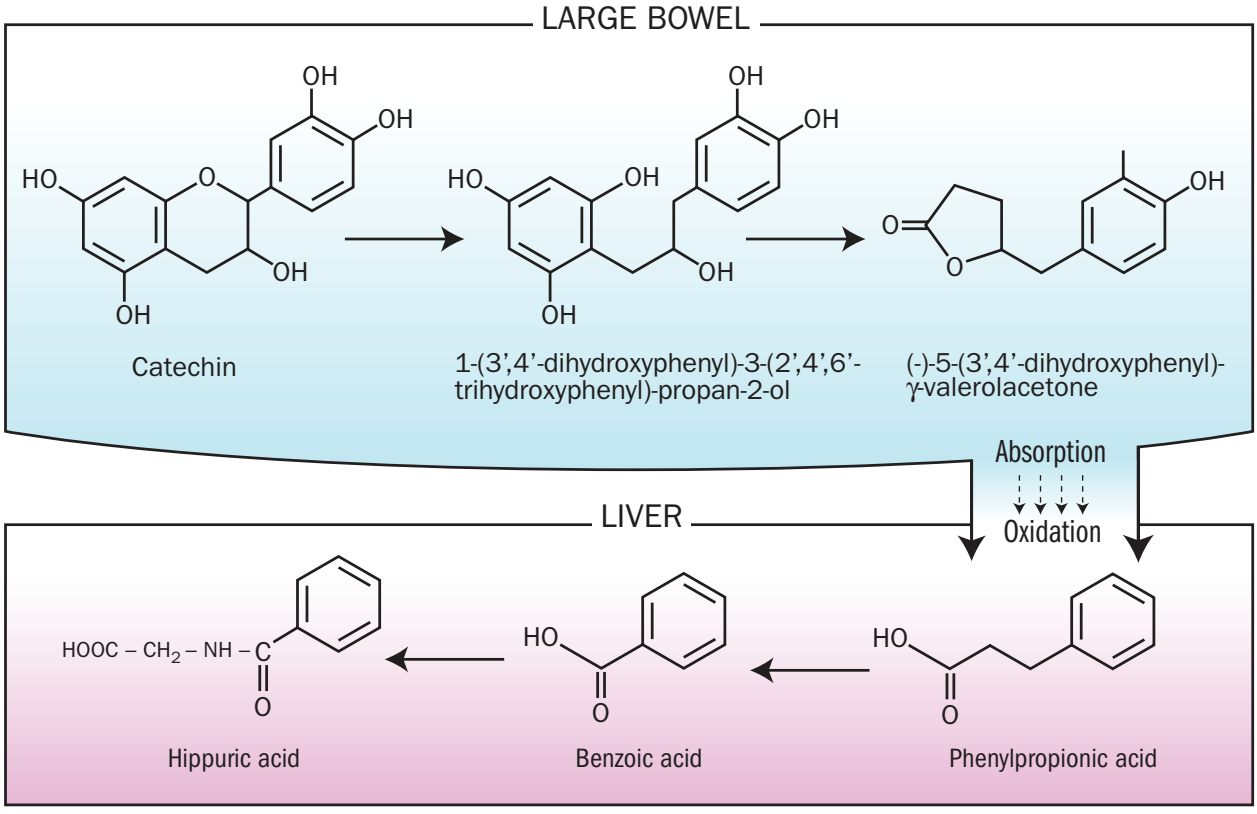
Figure 1. Bacterial and Human Enzymatic Conversion of Catechin to Hippurate



Products from Dietary Phenolic Compounds

Benzoate and Hippurate

Benzoate was one of the compounds first found to be elevated in urine from patients with intestinal bacterial overgrowth of various origins. Many patients with intestinal bacterial overgrowth resulting from cystic fibrosis, unclassified enteritis, celiac disease, or short bowel syndrome were found to have elevated benzoate along with various degrees of elevated phenylacetate, p-hydroxybenzoate, and p-hydroxyphenylacetate, as described below.[4] These products were thought to be derived from unabsorbed phenylalanine or tyrosine released from dietary protein.[4] Later reports demonstrated bacterial catabolism of dietary polyphenols may be the predominant origin of benzoate, which is normally conjugated with glycine in the liver to form hippurate.[5] Dietary polyphenols generally persist into the lower small intestine because they are resistant to degradation by digestive fluids.[6]

Coffee, fruits, and vegetables are sources of the polyphenolic chlorogenic acid, over 57 percent of which is recovered in urine as organic acids, mainly benzoate and hippurate.[7] Caffeine is also present in coffee and tea, but is not a polyphenolic compound and does not yield any of the compounds currently measured as intestinal microbial products in urine. Quinic acid, a tetrahydroxybenzoic acid compound found in tea, coffee, fruits, and vegetables, is also largely metabolized to benzoic acid by intestinal bacteria and excreted as hippurate.[8] When humans were experimentally switched from a low-polyphenol diet to one including 6 g of green tea or black tea solids, they began excreting more hippurate.[9] Consumption of 6 g of green tea solids by healthy male volunteers produced an approximate doubling of urinary hippurate, from 1.9 to 4.0 mmol/24 hours.[9] A change of 2 mmol/24 hours corresponds to

Table 1. Interpretation of Urinary Benzoate and Hippurate Abnormality Patterns

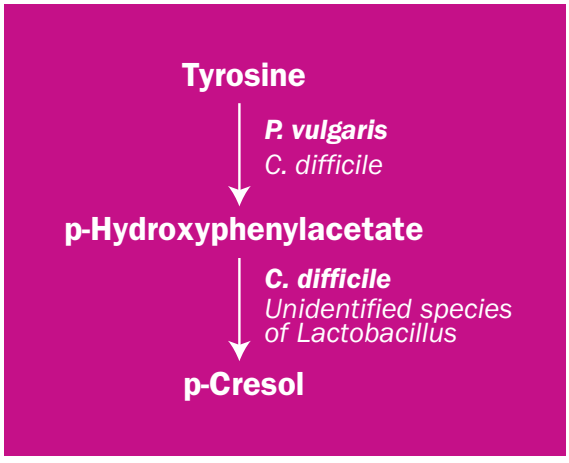
Benzoate	Hippurate	Other Bacterial Markers	Interpretation
Low	Low	No elevations	Low intake of benzoate and precursors, plus normal or low dietary polyphenol conversion by intestinal microbes
		Multiple elevations	Low intake of benzoate and precursors with intestinal microbial overgrowth of species that do not metabolize dietary polyphenols (very rare)
High	Low	No elevations	Glycine conjugation deficit (possibly genetic polymorphic phenotype if hippurate is very low); dietary benzoate or precursor intake
		Multiple elevations	Glycine conjugation deficit; presume benzoate is at least partially from intestinal microbial action on dietary polyphenols
Low	High	No elevations	Normal hippurate production via active glycine conjugation; No indication of microbial overgrowth
		Multiple elevations	Normal hippurate production via active glycine conjugation; Presume hippurate is at least partially derived from intestinal microbial action on dietary polyphenols
High	High	No elevations	Very high dietary benzoate or precursor intake with partial conversion to hippurate
		Multiple elevations	Very high benzoate load, some, or all, of which is contributed by intestinal microbial action on dietary polyphenols

approximately 120 mcg hippurate/mg creatinine. The effect from normal tea consumption is small compared with a typical abnormal cutoff of about 1,000 mcg hippurate/mg creatinine. Similar results from ingestion of brewed black tea were reported prior to the tea extract study.[10] In addition, other studies found the measured levels of phenolic compounds from green tea are lowered by administration of an antibacterial agent to a human subject, confirming the microbial contribution to the appearance of urinary products.[11] Figure 1 shows the overall bacterial and hepatic conversion of catechin (a principal phenolic compound from tea leaves) to hippurate.

Benzoic acid is also a common food component. It is used as a preservative in packaged foods such as pickles and lunch meats, and occurs naturally in cranberries and other fruits.[12] This should be taken into account when interpreting elevated hippurate levels in urine. Whether the source is dietary intake or jejunal bacterial metabolism, benzoate should be rapidly converted to hippurate by conjugation with glycine. Glycine and pantothenic acid can be limiting factors in this process. Availability of glycine is easily limited as discussed in part 1 of this series.[13] Elevated benzoate is a confirmatory marker for inadequacy of glycine or pantothenic acid for conjugation reactions in the detoxification system.[14,15] Abnormalities of urinary benzoate and hippurate may reveal clinically significant detoxification or dysbiosis issues. High benzoate indicates poor detoxification via phase II glycine conjugation. Interpretations of other scenarios are collected in Table 1.

The organic solvent toluene is metabolized by oxidation to benzoic acid and excreted as hippurate.[16] Although some reports have associated hippurate excretion with exposure to toluene, the relationship is weak because of the multiple other sources of hippurate described here. Short-term toluene exposure produces no significant change in hippurate excretion.[16]

Figure 2. Cresol from Bacterial Metabolism of Tyrosine



Phenylacetate and Phenylpropionate

Urinary phenylacetic acid (PAA) is the product of unidentified, specific strains of bacteria, marking a state of bacterial overgrowth when it is elevated in urine. Intestinal bacterial action on dietary polyphenols causes the appearance of PAA in urine. Excretion of PAA is markedly increased after the gastrointestinal tracts of germ-free rats are inoculated with fecal microorganisms, indicating its microbial origin.[5] Significant PAA has been found in human fecal water, indicating absorption from the gut is only partial, which has potential implications for involvement in colonic function.[17] For individuals with normal, healthy intestinal function, phenylacetate should not appear in more than background concentrations in urine. However, phenylacetate is a trace product of endogenous phenylalanine catabolism that can accumulate in the phenylalaninemic state found in phenylketonuria (PKU).[18] Although PAA shows little toxic effect on brain glutamatergic activity,[19] it has significant effects on hepatic flux of glutamate and α-ketoglutarate,[20] indicating PAA may mediate some of the toxic consequences of PKU.

Phenylpropionic acid (PPA), a compound similar to PAA with two $-CH_2-$ groups instead of one, is also produced by anaerobic gut flora.[21] PPA does not normally appear in human urine, however, because it is metabolized by mitochondrial medium-chain acyl-CoA-dehydrogenase (MCAD).[22] The glycine conjugate of PPA, 3-phenylpropionylglycine, has been proposed as a marker for diagnosing asymptomatic MCAD-deficient individuals who do not sufficiently carry out the oxidative step.[23] This human genetic polymorphism test is unique in being dependent on the production of PPA by gut flora such as *Peptostreptococcus anaerobius*. Because of the intestinal bacterial requirement, the question of which organisms may be required has been addressed in one study. Of 67 bacterial and five yeast isolates examined, only the three isolates of *Clostridium sporogenes* and one of *Clostridium difficile* produced PPA.[24]

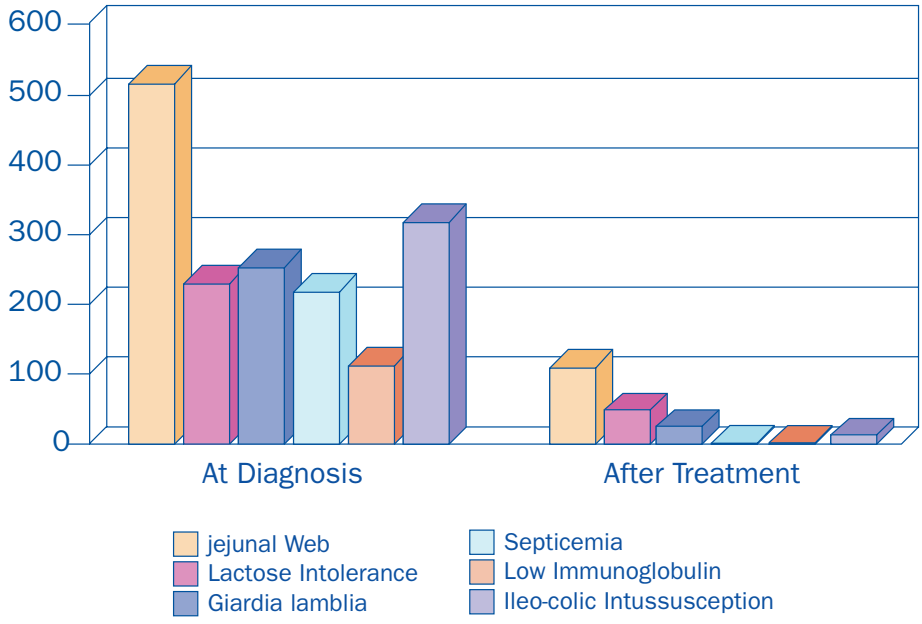
Cresol and Hydroxybenzoate

Dietary polyphenols or tyrosine residues from dietary proteins are compounds from which urinary p-cresol, p-hydroxybenzoate, and p-hydroxyphenylacetate are formed (Figure 2).[25] Cresol has a chemical structure very similar to phenol and is highly toxic. Cresol excretion is not affected by dietary protein intake, suggesting the bacteria responsible reside in the lower portions of the small intestine where amino acids from dietary protein rarely penetrate. These bacteria apparently produce cresol from intestinal secretions as well as from dietary sources.[26] Mammalian tissues have negligible metabolic activity toward absorbed cresol according to studies in sheep, where 95 percent of cresol infused into the rumen appears in urine.[25] Production of cresol in humans may be dependent on small intestinal populations of aerobic or microaerophilic bacteria because, in sheep, its production is almost exclusively confined to the rumen.[27]

A large majority of adult celiac disease patients were found to excrete unusually high amounts of p-cresol.[28] Due to the loss of renal function, uremic patients accumulate cresol, which may contribute to toxic effects. The resultant increase in serum cresol can be prevented by the use of non-absorbed oral sorbents, demonstrating the origin of p-cresol is the bowel. Finely powdered, activated charcoal is a generally available sorbent, but newer synthetic compounds may also be effective.[29] Cresol excretion was found to be lowered by administration of prebiotic substrate (oligofructose-enriched inulin) along with *Lactobacillus casei* Shirota, and *Bifidobacterium breve* to human subjects.[30]

Strains of *Escherichia coli* can produce p-hydroxybenzoate from glucose.[31] Esters of p-hydroxybenzoate, called parabens, have antibacterial activity[32] and are part of the mechanism for establishing bacterial dominance in intestinal populations.

Figure 3. p-Hydroxyphenylacetate Response to Treatment



Hydroxyphenylacetate

Although no other species has a digestive tract exactly like humans, the one with the closest resemblance is swine. Studies in newly weaned pigs have revealed specific microbes that carry out tyrosine degradation (Figure 2).[33] Both the transamination to form p-hydroxyphenylacetate (HPA) and the decarboxylation to p-cresol are carried out by *Clostridium difficile*. Since *Proteus vulgaris* can do only the first of these steps, HPA will increase in urine if *P. vulgaris* is the predominant organism. When *P. vulgaris* is accompanied by overgrowth of a newly identified strain of Lactobacillus, however, p-cresol will be the major product to accumulate. Such studies illustrate the potential for more specific bacterial identifications based on patterns of products appearing in urine. To achieve more detailed assignments of origin, urine collections may need to be timed following intake of specific sources. For example, a bolus of black currant juice can cause the appearance of different products as it passes from one region of the gut to the next.[34]

p-Hydroxyphenylacetic aciduria has been found useful in detecting small bowel disease associated with *Giardia lamblia* infestation, ileal resection with blind loop, and other diseases of the small intestine associated with anaerobic bacterial overgrowth.[35] Use of antibiotics that act primarily against aerobic bacteria (such as neomycin) can encourage the growth of protozoa and anaerobic bacteria that then produce greater amounts of these compounds.[36] A clostridial species isolated from swine feces carries out the further metabolism of p-hydroxyphenylacetate to p-cresol.[33]

Patients with cystic fibrosis, or other conditions that severely impair amino acid absorption, can demonstrate the potential for intestinal bacterial conversion of phenylalanine and tyrosine to phenyl compounds that appear in urine. These patients tend to excrete very high levels of phenylacetate and HPA.[37] However, since tyrosine released from dietary protein is rapidly absorbed in most individuals, conversion of tyrosine to HPA may be a rarely observed sign of dysbiosis in humans. However, the other isomers, o- and m-hydroxyphenylacetate, may be derived from dietary polyphenols that are unaffected by digestive enzymes; they are normally abundant dietary components. Of the three isomers, the most likely bacterial dysbiosis marker is m-hydroxyphenylacetate, which appears when bacteria are introduced

to germ-free rats, and increases markedly when humans are fed catechin and proanthocyanidin-rich chocolate.[38] In experiments conducted with a human anaerobic fecal fermentation device, quercetin was found to be metabolized within two hours to 3,4-dihydroxyphenylacetate, which, over the next eight hours, was converted to m-hydroxyphenylacetate.[39]

HPA is elevated in a wide variety of conditions involving direct intestinal pathology or digestive organ failure (Figure 3), which have obvious potential for dysbiosis. Although treatments vary greatly depending on the nature of the disorder, the lowering of elevated urinary HPA reveals a normalized intestinal bacterial population.[4] Some microbial compounds are absorbed and enter the detoxification pathways of the liver to be excreted as modified products that can serve as indicators of gastrointestinal activities. For example, bacterial amines are converted to piperidine, a sensitive biochemical index of gastrointestinal flora changes in celiac disease.[40] Other compounds appear due to genetic traits that affect how bacterial products are metabolized. The anaerobic bacterial product, 3-phenylpropionate mentioned previously, for example, is normally converted to common hippuric acid, but is excreted as 3-phenylpropionylglycine in individuals with a relatively common inborn error of fatty acid oxidation.[24] Some compounds excreted in these instances are not organic acids, so they must be analyzed in separate assays to enhance the interpretation of origins for microbial compounds in urine.

Hydroxyphenylpropionate

The o- (or 2) and m- (or 3)-hydroxyphenylpropionates can reveal specific types of intestinal bacterial activity. When germ-free rats are given feed that is contaminated with feces from standard rats, they begin to excrete m-hydroxyphenylpropionate (m-HPPA).[5] Subsequent studies showed that m-HPPA is absent from the urine of germ-free rats, whereas it is the principal product that appears from conventional rats when caffeic acid is introduced.[41] Increased excretion of m-HPPA was found in healthy human volunteers who consumed 1,000 mg of polyphenols as grape seed extract.[42] Low levels of urinary m-HPPA, therefore, can indicate low intake of caffeic acid and the proanthocyanidins found in grapes and other foods. High levels of m-HPPA, on the other hand, may indicate increased intestinal bacterial metabolism of dietary catechins and caffeic acid. m-HPPA systematically increases in rat urine when catechin is added to their chow, and its excretion in urine drops from around 200 mcg/24 hours to 10 mcg/24 hours after administration of a combination sulfathiozole + auromycin antibiotic.[43] The data from the chlorogenic acid study reveals a wide range of individual variation in responses. Such responses could be attributed to variation in intestinal bacterial conversion potential from the normal rates exhibited by most people.

In vitro bacterial growth experiments indicate that, in the gut, the p- (or 4) isomer p-HPPA is metabolized by bacteria but not by protozoa. Bacterial action converts p-HPPA into p-hydroxybenzoate, p-hydroxyphenylacetate, phenylpropionate, phenyllactate, and phenylpyruvate.[44]

When p-HPPA is elevated without concurrent elevation of tyrosine, then the possibility of intestinal clostridial production from dietary tyrosine should be considered. Under *in vitro* conditions, where L-tyrosine is supplied as a growth substrate, p-HPPA is a major product of *Clostridium sporogenes*, *Clostridium botulinum A*, *C. botulinum B*, and *Clostridium caloritolerians*.[45] Such growth conditions also result in the appearance of even greater concentrations of phenylpropionate, but insignificant amounts of phenylacetate, phenyllactate, p-hydroxyphenylacetate, and indole. Human fecal bacteria grown with the polyphenol naringin as a substrate show predominant production of phenylpropionate or p-HPPA.[1] These results help to explain the varied patterns of urinary products that appear with individual patients.

Abnormal appearance of the o- or m- isomers indicates the more common bacterial overgrowth utilizing dietary polyphenols, whereas high p-hydroxyphenylacetate may be due to type III tyrosinemia or bacterial conversion of unabsorbed tyrosine. Patients with the genetic trait will present with characteristic signs of type III tyrosinemia, whereas those with chronic maldigestion of protein will generally show gastrointestinal signs.

3,4-Dihydroxyphenylpropionate

Several clostridial species are known to cause human disease, for example, *Clostridium difficile*-associated enteric disease epidemics[46] and *Clostridium perfringens* associated food borne infectious illness outbreaks from eating cooked beef.[47] However, many species of

the genus Clostridium make up a major portion of the bacterial population in the normal human gut, with *Clostridium coccoides* frequently found as the most abundant species.[48] The importance of Clostridia to urinary product formation is both their abundance and their metabolic diversity.

The full designation of the compound discussed here is 3-(3,4-dihydroxyphenyl)-propionic acid, which we shorten to 3,4-dihydroxyphenylpropionic acid and abbreviate as 3,4-DHPP. Numerous reports have been received of patients with Clostridium overgrowth confirmed by stool culture, where elevated levels of 3,4-DHPP have fallen to baseline with Flagyl, but were unaffected by nystatin.[46] Although other organisms may produce 3,4-DHPP, Clostridia is the most commonly encountered genera among those susceptible to Flagyl. *In vitro* studies have confirmed the production of 3,4-DHPP from dietary quinolines by various clostridial species.[49,50] Rats excrete 3,4-DHPP when they are fed the naturally occurring flavonoid hesperetin.[51] Depending on the species, Clostridia excrete various other organic acids as the end products of aromatic amino acid metabolism.[45] Cytotoxic quinoid metabolites that require glutathione conjugation for removal may be formed from 3,4-DHPP.[52] Various compounds closely related to 3,4-DHPP are also produced by the genus Clostridium.[45] In addition, 3,4-DHPP has been found as a product of metabolism of quinoline by *Pseudomonas stutzeri*.[53,54] Intestinal 3,4-DHPP is degraded by an enzyme produced by *E. coli*, thus helping to insure its survival in the presence of intestinal clostridial growth.[55,56]

Products from Tryptophan

Indican

Bacteria in the upper bowel produce the enzymes that catalyze the conversion of tryptophan to indole. Absorbed indole is converted in the liver to indoxyl, which is then sulfated to allow for urinary excretion. Indoxyl sulfate (also known as indican) can be measured colorimetrically by conversion to colored oxidation products or directly by liquid chromatography with a U.V. absorption or mass spectrometric detector.

Because the upper bowel is sparsely populated with bacteria, indican is present in urine at low levels in healthy individuals. An elevated level of urinary indican is an indication of upper bowel bacterial overgrowth. Certain patients, such as those with celiac disease, may be at greater risk.[28] Bacterial overgrowth utilizing urinary indican was demonstrated in eight of 12 patients following jejuno-ileal bypass surgery.[57]

Oral, unabsorbed antibiotics reduce indican excretion.[58] Indican excretion is also reduced when the gut is populated with strains of Lactobacillus at levels above 10^5 organisms/g.[58] *Lactobacillus salivarius*, *Lactobacillus plantarum*, and *Lactobacillus casei* were more effective in achieving reduced indican than two strains of *Lactobacillus acidophilus*. In patients with cirrhosis of the liver, tryptophan loading can produce neuropsychiatric manifestations due to intestinal bacterial production of tryptophan metabolites.[59] The symptoms are reduced by antibiotic therapy, demonstrating the bacterial origin of the metabolites.

Indican testing can aid in differentiating pancreatic insufficiency from biliary stasis as the cause of steatorrhea (fatty stools).[60] Patients with steatorrhea due to pancreatic insufficiency show a rise of indican from low values to above normal when they are treated with pancreatic enzyme extract.[60] Urinary indican does not rise in patients with steatorrhea not due to pancreatic insufficiency, nor in normal subjects who receive pancreatic enzymes. This scenario demonstrates how bacterial populations respond to increased concentrations of luminal amino acids. Large shifts in bacterial populations induced by the artificial sweetener saccharin have also been demonstrated by changes in indican excretion.[61]

No age adjustment for reference limits is necessary, since excretion has been shown to be constant for young and elderly control subjects.[62] The test sensitivity may be enhanced by oral loading of 5 g tryptophan.[63] The number of false-positives can be reduced by including elevations of other bacterial metabolites with that of indican as criteria for abnormal bacterial colonization of the small intestine.[64]

The interpretation of indican results is complicated by impaired protein digestion, which increases the tryptophan available for bacterial action. Even patients with normal intestinal bacterial populations can show increased postprandial indican excretion when they fail to digest dietary protein. The relationship between increased indican and incomplete digestion might be utilized as a measure of protein digestive adequacy. Indican evaluation has been used to assess intestinal absorption

Table 2. Neurological Signs and Symptoms in 29 Patients with D-Lactic Acidosis[80]

Symptom	% of Patients
Altered mental status ranging from drowsiness to coma	100
Slurred speech	65
Disorientation	21
Impaired motor coordination	21
Hostile, aggressive, abusive behavior	17
Inability to concentrate	14
Nystagmus	14
Delirium	10
Hallucinations	10
Irritability	3
Excessive hunger	3
Headache	3
Partial ptosis	3
Asterixis	3
Blurred vision	3

of tryptophan in scleroderma.[65] Increased urinary indican has been shown to correlate with enteric protein loss.[66] Indican elevation has revealed that impaired protein digestion and increased bacterial conversion of tryptophan is a complication of cirrhosis of the liver.[67] Some degree of malabsorption was found in 30 percent of an elderly population by combinations of indican with the Shilling and other tests.[68]

Products of Dietary Carbohydrate

D-Lactate

Although nanomolar concentrations of D-lactic acid may be produced by human tissues,[69] it is a major metabolic product of several strains of bacteria that inhabit the human gut.[70] D-lactate is frequently detected in patients with short-bowel syndrome, due to poor dietary carbohydrate absorption because of impaired absorptive regions in the upper small intestine. Many genres of bacteria can convert simple sugars into D-lactate. However, *Lactobacillus acidophilus* is uniquely adapted to withstand the dramatically lowered intestinal pH resulting from massive accumulation of luminal D-lactate and other organic acids. Under conditions of carbohydrate malabsorption, D-lactate is simultaneously increased in blood and urine.[71] Some D-lactate entering portal circulation can undergo hepatic conversion to carbon dioxide, but this pathway has limited capacity. This limitation is in contrast to the extremely large capacity for metabolism of the L-lactate isomer produced in skeletal muscle and other tissues. With continued increases in intestinal output, rising blood levels are reflected in urinary output of D-lactate.[72] When intestinal production rates exceed the capacity for clearance, D-lactic acidosis is produced.[73] Intestinal symptoms of diarrhea are frequently present due to the disruption of bowel flora.[74,75]

D-lactic acidosis due to overgrowth of *Lactobacillus plantarum* was reported in a child who developed an unusual encephalopathic syndrome due to neurotoxic effects of D-lactate.[76] D-lactic acidosis may be accompanied by any of the various neurological symptoms listed in Table 2.[71,77,78] Attacks are usually episodic, lasting from a few hours to several days. Direct toxic effects of D-lactate in the brain are suspected.[77,79]

Jejuno-ileostomy patients have the highest risk of developing D-lactic acidosis and accompanying encephalopathy because they usually have some degree of carbohydrate malabsorption.[81,82] Procedures as mild as stomach stapling may lead to D-lactic acidosis.[73] Precipitating factors include use of antibiotics[83] and medium-chain triglycerides.[84] Carbohydrate malabsorption associated with pancreatic insufficiency can also induce D-lactic acidosis.[85] Elevated levels of D-lactate were found in blood samples of 13 of 470 randomly selected hospitalized patients.[86] Studies in cattle have confirmed that increases in D-lactate following overloading of grain in the diet corresponded to growth of Lactobacilli rather than coliform bacteria.[87]

The specificity and sensitivity of urinary D-lactate has led to the test being proposed for routine diagnosis of bacterial infections.[88] D-lactate has also been reported to be a marker for diagnosis of acute appendicitis,[89] and for differentiating perforated from simple appendicitis.[90] Whatever the origin, patients are managed with antibiotics and probiotics,[91] including *Saccharomyces boulardii*.[71]

During acidotic episodes in patients with short-bowel syndrome, 24-hour urinary excretion of D-lactate can rise to levels above 600 mcg/mg creatinine, far higher

Table 3. Lactate Isomers Produced by Individual Species of Lactobacillus[94]

Producers of Only D(-)-Lactate	Producers of Only L(+)-Lactate	Producers of Racemate DL-Lactate
Lactobacillus delbrueckii subsp. delbrueckii	*Lactobacillus agilis*	*Lactobacillus acidophilus*
Lactobacillus delbrueckii subsp. lactis	*Lactobacillus amylophilus*	*Lactobacillus amyiovorus*
Lactobacillus delbrueckii subsp. bulgaricus	*Lactobacillus animalis*	*Lactobacillus aviarius subsp. aviarius*
Lactobacillus jensenii	*Lactobacillus bavaricus*	*Lactobacillus brevis*
Lactobacillus vitulinus	*Lactobacillus casei*	*Lactobacillus buchnari*
	Lactobacillus mali	*Lactobacillus crispatus*
	Lactobacillus maltaromicus	*Lactobacillus curvanus*
	Lactobacillus murinus	*Lactobacillus formentum*
	Lactobacillus paracasei subsp. paracasei	*Lactobacillus gasseri*
	Lactobacillus paracasei subsp. tolerans	*Lactobacillus graminis*
	Lactobacillus ruminis	*Lactobacillus hamsteri*
	Lactobacillus salivarius	*Lactobacillus helviticus*
	Lactobacillus sharpeae	*Lactobacillus homohiochii*
	Lactobacillus rhamnosus	*Lactobacillus pentosus*
		Lactobacillus plantarum
		Lactobacillus reuteri
		Lactobacillus sake

than concurrent L-lactate concentrations of around 24 mcg/mg creatinine.[78] D-lactic acidosis has also been reported in a patient with chronic pancreatitis and renal failure.[85] Compared to controls, significant elevations of D-lactate were reported for ischemic bowel, small bowel obstruction, and acute abdomen, with a negative predictive value of 96 percent and a positive predictive value of 70 percent.[92]

The phenomenon of D-lactic acidosis has been described as turning sugar into acid in the gastrointestinal tract.[93] D-lactate is not the only organic acid produced from simple carbohydrates. Although carbohydrates are also turned into p-hydroxybenzoate and tricarballylate, those compounds are never absorbed at rates that can produce the systemic effects found with D-lactate. When D-lactate is elevated, supplementation with D-lactate-producing species of Lactobacillus is contraindicated, and steps to reduce bacterial populations should be considered. Not all species of Lactobacillus produce significant D-lactate, as shown in Table 3. Once the carbohydrate excess in the small intestine is controlled, a recommended approach to managing recolonization with probiotic species is to supplement with species that do not produce D-lactate.

Urinary D-lactate reference values of 5.9 and 13.7 mcg/mg creatinine for adults and children less than one year old, respectively, have been reported.[77,78,95] Studies that have performed simultaneous plasma and urine specimen collections show that urinary concentrations can frequently be 10-fold higher than plasma.[91] An advance in analytical sensitivity has recently been achieved in which a single chiral chromatographic separation allows resolution and low-level accuracy for simultaneous, quantitative analysis of D- and L-lactate by tandem mass spectroscopy.[96] Since independent enzymatic methods frequently have varying calibration errors and efficiencies of recovery, the simultaneous determination of both isomers allows more accurate detection of patients predominantly excreting the D-isomer. In summary, urinary D-lactate elevation may predict bacterial

overgrowth as a result of: carbohydrate malabsorption, ischemic bowel, certain types of pancreatic insufficiency, acute appendicitis, and surgical procedures that compromise upper gastrointestinal function. Diagnosis and treatment of D-lactic acidosis can significantly improve patient outcomes.

Tricarballylate

Tricarballylate (tricarb) is produced by a strain of aerobic bacteria that quickly repopulates in the gut of germ-free animals.[97] As its name implies, tricarb contains three carboxylic acid groups that are ionized at physiological pH to give a small molecule with three negative charges, akin to the structure of the powerful chelating agent EDTA. Magnesium is bound so tightly by tricarb that magnesium deficiency results from overgrowth of tricarb-producing intestinal bacteria in ruminants.[98] This condition, known as "grass tetany," is also accompanied by lower levels of calcium and zinc, all of which can form divalent ion complexes with tricarb.

Products of Fungi (Yeast)

D-Arabinitol

D-arabinitol (DA) is a metabolite of most pathogenic Candida species, *in vitro* as well as *in vivo*. D-arabinitol is a five-carbon sugar alcohol that can be assayed by enzymatic analysis. It is important to distinguish the sugar alcohol from the sugar D-arabinose that is unrelated to any yeast or fungal condition in humans. A single report of two autistic brothers who were found to have significant concentrations of arabinose in their urine has led to claims about possible associations of yeast infections and autism,[99] although no further evidence in support of this association has been reported. DA, on the other hand, has long been known to be associated with candidiasis in a variety of clinical situations.[100-102] The enzymatic method using D-arabinitol dehydrogenase is precise (mean intra-assay coefficients of variation [CVs], 0.8%, and mean interassay CVs, 1.6%), and it shows excellent recovery of added DA.[103]

Among pathogenic yeasts and fungi, Candida spp. are of widest clinical concern, because of their transmission by direct invasion of the gastrointestinal and genitourinary tracts and their ability to rapidly overwhelm immune responses in many hospitalized patients. Most species of Candida grow best on carbohydrate substrates. Activities of the enzymes aldose reductase and xylitol dehydrogenase are induced in Candida tenuis when the organism is grown on arabinose.[104] The rate of DA appearance in the body equals the urinary excretion rate and is directly proportional to the concentration ratio of DA to creatinine in serum or urine.[105]

Measuring serum DA allows prompt diagnosis of invasive candidiasis.[106] Immunocompromised patients with invasive candidiasis have elevated DA/creatine ratios in urine. Positive DA results have been obtained several days to weeks before positive blood cultures, and the normalization of DA levels correlate with therapeutic response in both humans and animals.[107,108] Elevated DA/creatinine ratios were reported in 69-, 36-, and nine-percent of patients with Candida sepsis, Candida colonization, and bacterial sepsis, respectively.[109] In another study, when patients were divided into categories of superficial candidiasis; possible deep, invasive candidiasis; and definite, deep invasive candidiasis, all three groups showed significant DA elevations.[110] Another group reported highly elevated, slightly elevated, and normal DA levels in two, two, and three patients, respectively, with superficial Candida colonization.[111] Yet a fourth independent group reported the appearance of DA in both disseminated and simple peripheral candidiasis.[112] The somewhat more discriminating elevated urine D-arabinitol/L-arabinitol (DA/LA) ratio has been found to be a sensitive diagnostic marker for invasive candidiasis in infants treated in neonatal intensive care units. Eight infants with mucocutaneous candidiasis were given empiric antifungal treatment, but had negative cultures; five of these had repeatedly elevated DA/LA ratios. Three infants with suspected and four with confirmed invasive candidiasis experienced normalized ratios during antifungal treatment.[113] The ratio of D- to L-arabinitol in serum reveals the presence of disseminated candidiasis in immunosuppressed patients.[108]

Putative Yeast Markers and Bacterial Markers

Tartarate, citramalate, and other compounds were found at high concentrations in two brothers who had conditions thought to be associated with intestinal yeast overgrowth.[99] Based on this anecdotal evidence alone, extensive misinformation has been disseminated about the significance of finding these compounds in urine. There is no reason to suspect intestinal yeast as the origin of any levels of these compounds in human urine. No evidence has appeared to support the contention that tartarate or citramalate are products of intestinal yeast overgrowth. Furthermore, the large dietary intake effects on urinary tartarate were not controlled in the single previous study.[114] D-arabinitol is the only urinary biomarker of invasive Candida sp. overgrowth that has reliable scientific support.

Early studies on bacterial isolates showed various strains of coliform bacteria can decarboxylate amino acids to their amine forms. Thus, *Bacterium coli* decarboxylated arginine, lysine, ornithine, histidine, and glutamic acids to agmatine, cadaverine, putrescine, histamine, and γ-aminobutyric acids, respectively.[115-119] These data must be viewed with caution, however, because they do not reveal the extent to which the products may be further metabolized by other microbial species in the gut or by human tissues. Although the urinary amino acid product γ-aminobutyrate is sometimes referred to as a marker of intestinal bacterial overgrowth, there is little direct or indirect evidence to support such a claim.

Conclusion

Elevated levels of specific organic acids in human urine may indicate abnormal rates of intestinal microbial growth. The anatomical location of the overgrowth is usually the mid-gut region that is most difficult to examine or directly assay for microbial activity by other means. Finding elevations of microbial-specific products can lead to improved patient outcomes when appropriate therapy is implemented to normalize the number and type of bacteria that may be proliferating in the small intestine.

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