URINARY ORGANIC METABOLITES

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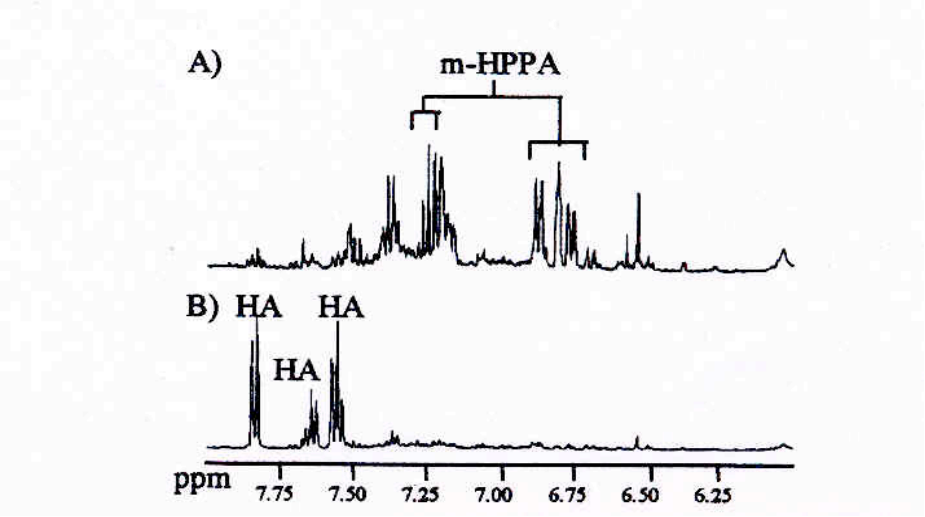
Introduction

Studies of gas chromatography/mass spectrometry (GC-MS) techniques carried out in 1994 on human clinical samples had already suggested a "potential for diagnosis and studies of metabolism *in situ*" [1]. Researchers found that, when this technique is applied to blood or urine, it gives an insight into the infected host microbial metabolism [1]. A more recent study (2002) on urinary organic metabolites, published in *Xenobiotic*, most convincingly provides evidence that the level of several urinary metabolites can be affected by differences in the metabolic capability of the intestinal microflora [2].

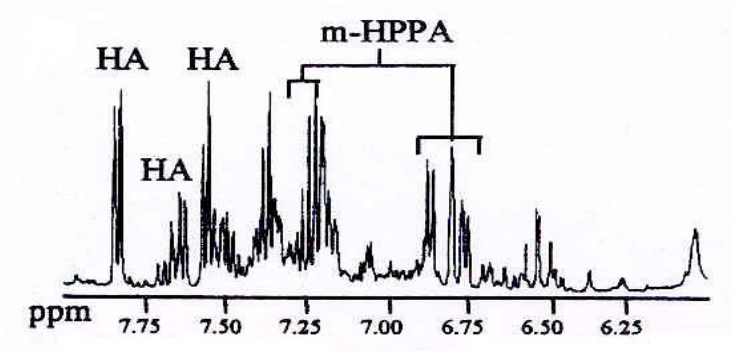
Central Toxicology Laboratory Animal Study

This animal study took place in Macclesfield (UK), at the Central Toxicology Laboratory (CTL) in Alderley Park. The sample constituted two groups of rats of an identical genetic lineage. To prevent any bacterial cross-contamination, the two groups of rats were housed under the same environmental conditions on two different floors of a barriered breeding unit, both groups being fed the same batch of diet [2].

Surprisingly, the tests showed that the animals had different urinary metabolite profiles. Rats from group "A" were predominantly excreting ***methyl-hydroxyphenyl-propionic acid*** (***m-HPPA***) while rats from group "B" were excreting predominantly ***hippuric acid*** (***HA***) [2] (fig.1):

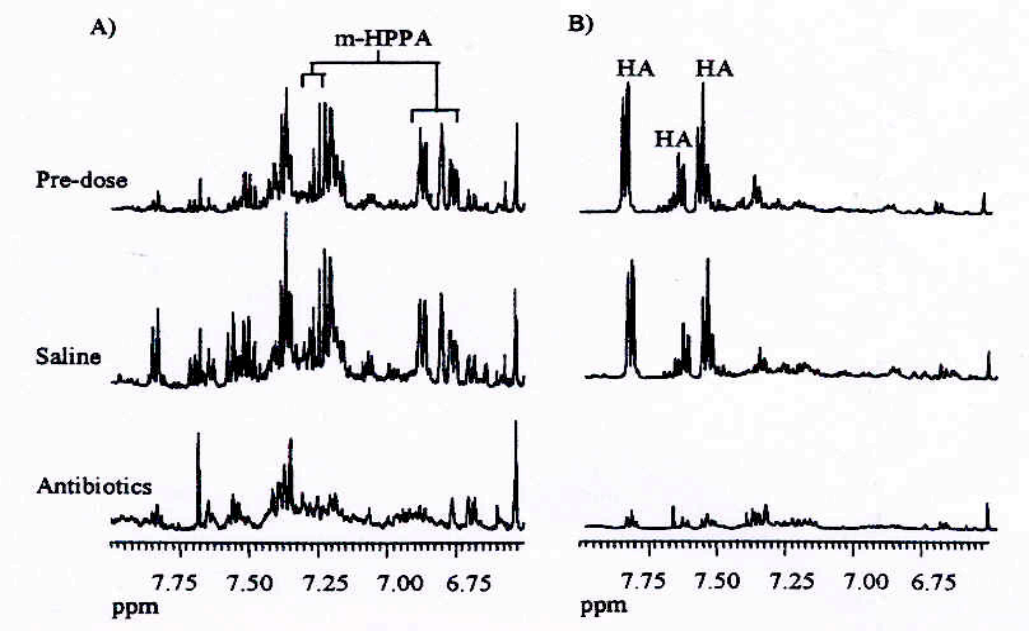


"Enquiries into the origin of the animals revealed that the animals that excreted ***m-HPPA*** originated from a different floor within the breeding unit than those animals that excreted ***HA***". However, when animals from each floor were housed together, the excretion profiles became harmonised after only 7 days [2] (fig. 2):



Removing the animals from the environment of their barriered breeding units allowed bacterial species present in their intestines to populate all animals. In fact, a previous study published in 1997 by a Japanese team [3], had already demonstrated the "infectious nature" of intestinal bacteria. In conclusion, we can assume that "profile harmonisation due to contact between the two groups of animals supports [the view] that differences in intestinal microflora might be responsible for the distinct profiles" [2].

This view is strengthened by the results of the evolution of those urinary metabolites when the rats were treated with either saline or antibiotics. The antibiotic regimen suppressed the bacterial growth and its consequences on the profiles [2] (fig. 3):



An article published in *Nature Review of Microbiology* in May 2005 suggested the use of "the metabolite signature that is found in host fluids such as urine" to improve "the understanding of dysbiosis and gut micro-organism related diseases processes" [4]. The researcher JK Nicholson stated that "several papers have detailed the identification of dynamic changes in the urinary levels of microbiotal products" in germ-free rats (bred and kept in a micro-organism free environment) as well as in conventional rats after they have been treated with antibiotics [4].

The evolution of urinary organic metabolite profiles of germ-free rats has been studied while the animals were acclimatised to standard (not sterile) laboratory conditions. The associated development of changes in the gut microfloral communities can easily be observed by following the corresponding urinary profiles over a three week-period, the time needed by the gut microflora to stabilise [5].

Study on Healthy Volunteers and Chamomile Tea

Similar tests were applied to humans in relation to their response to chamomile tea ingestion. A single cup of chamomile tea was given every day to a group of healthy volunteers for a 10 day period. When their urine was tested at the end of this period, the samples showed modifications in microbial metabolites. Chamomile tea was then withdrawn and their urine retested after a further 10 day period. It was found that the metabolic signature had not reverted to its pre-dose condition, thus indicating that the chamomile tea had a rather profound and not easily reversible effect on the gut flora [6].

This surprising result suggests that chamomile tea reselects microbial populations in the human gut probably through a weak but selective antibiotic action [6]. In fact, chamomile oil antibacterial properties towards *Helicobacter pylori* have been well documented, inhibiting bacterial growth "in extraordinarily low concentrations of 0.0075 %" [7].

Urinary Metabolites Provide Fungal Markers

Hence it could be possible to spot several fungal metabolites, such as *arabinose* and *arabinitol*, in the urine of patients suspected to suffer from intestinal fungal overgrowth. *D-arabinitol* is a typical metabolite of *Candida albicans* [8]. Its identification in the urine provides a clue to both the existence of candidiasis and its severity, giving us important qualitative and quantitative data [1]. We must emphasise that the urinary metabolite signature evaluates microorganisms *in situ* and therefore prevents the unsatisfactory flaws rising from false positive and, especially, false negative results linked to stool cultures.

Indeed, the main problem with stool cultures lies in the huge differences between the laboratory and the intestinal environmental conditions. Yeasts do not feel comfortable once excreted in the stools and many die or do not remain healthy enough to grow into observable colonies in the laboratory Petri dishes.

Besides, most gut bacterial species do not tolerate oxygen, many of them being strictly anaerobic. Indeed, the large majority of the intestinal microflora has either never been cultured or cannot be cultured in standard conditions [9].

Urinary Metabolites Provide Putrefactive Bacterial Markers

Multiple markers can be obtained from both the presence and the overgrowth of a typical putrefactive bacterial genus. For example, *Clostridium* metabolises the aromatic amino acids phenylalanine and tyrosine into phenolic compounds, and tryptophan into indolic compounds [10-12]. In 1979, Chalmers (a pioneer in genetic testing on urinary organic acids) had already measured ***4-hydroxyphenyl-acetic acid*** in urine as a screening method for small-bowel diseases and bacterial overgrowth syndromes [13].

"***Phenol***, ***para-cresol*** and hydroxylated phenol-substituted fatty acids are known to be the main products of tyrosine fermentation in anaerobic bacteria, whereas ***phenyl-acetate*** and ***phenyl-propionate*** are formed from phenylalanine. (…) ***Indole***, ***indole-acetate***, and ***indole-propionate*** are all products of tryptophan metabolism" [11]. It might even be possible, in the future, to identify the species of *Clostridium* according to their specific profiles of urinary organic metabolites [9]. *Clostridia* represent a putrefactive genus, thus all the above-mentioned urinary metabolites will reflect an overgrowth of putrefactive bacteria. Test profiles of urinary organic acids associated with putrefactive microbial overgrowth include ***benzoate***, ***hippurate***, ***phenyl-acetate***, ***phenyl-propionate***, ***cresol***, ***hydroxy-benzoate***, ***hydroxy-phenyl-acetate***, ***hydroxy-phenyl-propionate***, ***3,4-dihydroxy-phenyl-propionate***, and ***indican*** [14].

Urinary Metabolites Provide Fermentative Bacterial Markers

Human tissues can produce ***D-lactic acid*** in extremely small amounts (in fact only nanomolecular concentrations), whereas it represents a major metabolic product of several bacterial strains within the human gut [14]. Multiple strains secreting ***D-lactate*** belong to fermentative species, many of them within the genus *Lactobacillus* [15].

A study published in 1991 had already shown that ***tricarballylate*** is another metabolite produced by intestinal bacteria, as it quickly appears when a conventional intestinal microflora is implanted in germ-free rats [16]. ***Tricarballylate*** is produced by aerobic bacteria that repopulate rat intestines, and it is considered as a fermentative marker [16]. Interestingly, an excessive production of ***tricarballylate*** has been blamed for the cause of magnesium deficiency known as grass tetany in ruminants [17]. This phenomenon results from the molecular structure: it contains 3 carboxylic groups ionized at gut physiological pH that can bind magnesium very tightly, triggering the magnesium malabsorption [14].

An Everyday Diagnosis

On a practical level, sophisticated databases and experienced chromatography teams are much needed as more than 250 urinary organic metabolites "are either typically present or may be encountered in [human] urine" [18]. Fortunately for the patients, "a random specimen, preferably the first morning voiding when applicable, is an acceptable alternative" to the 24 hours urine collection [18].

Given that fluctuations on the ranges of excretion "mainly depend on individual metabolic variations rather than on dietary factors" [19], no special diet is needed prior to the collection of the first morning urine sample. However, with a complete change of diet, significant metabolite variations will ultimately occur, but the shift will take some time.

Conclusion

Intestinal microbial growth is accompanied by the release of products of their metabolism that tend to be absorbed by the intestinal lining and thereafter excreted in urine [14]. As a consequence, "*detection of abnormally elevated levels of these products is a useful diagnostic tool for patients with gastrointestinal and toxicological symptoms*" [14].

Dysbiotic patients can benefit from an easy-to-collect urine test that will facilitate the identification of fungal and/or bacterial overgrowths. Besides, the increase of specific markers can show-up overgrowths of either putrefactive or fermentative bacterial strains.

Moreover, these urinary organic metabolite profiles provide an insight not only into the existence but also into the severity of imbalances in the intestinal microflora, as they give quantitative measurements. This represents another crucial advantage comparatively to stool cultures, as you would not assume that laboratory Petri dishes ensure growths proportional to what effectively occurs in human intestines, if the microbes grow at all...

Perhaps the best proof that this diagnostic method gains popularity is a very recent article published in May 2010 about using urinary metabolic phenotyping to differentiate autistic children from their unaffected siblings and controls [20]. Differences in urinary metabolites (including ***hippurate***) are observed between autistic and control children [20], whereas autism has been associated with intestinal dysbiosis and with *Clostridium* overgrowth [21].

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