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Colonic Fermentation – more than meets the nose.

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Abstract

Fermentation of undigested foods in the colon by its resident bacteria affects not only colonic health (protection against inflammation and tumour formation) but also influences metabolic health. Studying fermentation directly is difficult for lack of access. We hypothesise that the anatomical structure of the colon is suited to act as a fermenting chamber with the gaseous molecules (VOCs) emitted having direct effects on the colonocytes as well as gut neural and metabolic effects. We refer to this complex system as the 'fermentome', and further hypothesise that alteration in the 'fermentome' through dietary modification will have a direct impact on colonic as well as metabolic health and disease. The VOCs emitted may play a role in bacterial chemical signalling within the colon but importantly could also function as a 'gas' biomarker. Measurement of such VOCs through non-invasive methods would have important application as a hypothesis-generating tool with subsequent clinical application.

Introduction

Colonic fermentation in man is commonly viewed as removal of "waste", and without further physiological significance. We propose instead that the gas products of fermentation may prove important for homeostasis through colonic bacterial chemical signalling and, by serving as a bio-transmitter(s), influence smooth muscle function. The gas is composed of various volatile organic compounds (VOCs), the relative proportions of which may change in disease. The study of such a 'fermentome' could then be used for diagnosis and disease characterisation. We have coined the term 'fermentome' to describe the complex interplay between diet, symbiont bacteria and volatile gases.

The Hypothesis

1) The distinct anatomical structure of the colon in humans is suited to act as a fermenting chamber. The gaseous molecules (VOCs) emitted as a result of fermentation will thus have direct effects on the colonocytes as well as gut neural and metabolic effects.
2) Alteration in the 'fermentome' through dietary modification will have a direct impact on colonic as well as metabolic health and disease.

Colonic gas: Physiological bio-transmitters?

Ethylene released from bananas regulates their ripening, an example from daily life of a gas bio-transmitter with powerful actions. In mammals, a paradigm shift from hormones acting as mediators or neurotransmitters evolved with the discovery that inorganic molecules mediate important physiological function. Examples include nitric oxide (NO): a powerful arterial tone regulator, it is now recognised to maintain gut mucosal integrity. Hydrogen sulphide (H_2S) inhibits pancreatic insulin release (by activating K_{ATP} channels in ß-cells), whilst in the gut it plays a pro-inflammatory role [1,2]. Thus, gaseous bio-transmitters may have several yet-undiscovered important physiological roles, which conceivably could be disturbed leading to disease.

Endogenous Gas and Fermentation

The concept of fermentation in human ecology is not new; the ancient Hebrews learnt that the rudimentary use of yeast in flour made all the difference to the end product, i.e. leavened bread. Since then its use has evolved in other areas, for example the wine and beer industry. Yet only recently has there been renewed interest in this biochemical reaction occurring in both prokaryotic and eukaryotic cells. Fermentation is defined as the process of deriving energy through oxidation of organic compounds such as sugars (carbohydrates). Emerging evidence suggests that fermentation of undigested foods in the colon by its resident bacteria (which are an order of magnitude greater than human cells collectively) influences colonic health through protection against inflammation and tumour formation. Colonic fermentation is regulated by the triad encompassing the colonocyte (constant), colonic bacteria (slightly variable) and diet (highly variable). Hence variation in diet is likely to have a significant impact on colonic fermentation and, to a lesser extent, on resident colonic bacteria. Endogenous intestinal bacteria also have an important fundamental role: acting in concert with the colonocyte they protect against cell injury, provide nourishment and inculcate innate immunity [3]. Thus, the endogenous production of gases as a result of fermentation is likely to be important in influencing general health.

Evidence of mal-fermentation in human disease?

Examples of possible mal-fermentation include 1) Observations of reduced incidence of inflammatory bowel disease (IBD) in Asian subjects but an increased incidence in the second generation who have adopted a 'western lifestyle', including a western diet [4]. Marchesi et al [5] have been able to demonstrate depletion of certain microbial-related metabolites in the faeces of patients with IBD, suggesting disruption of gut bacterial ecology and perhaps fermentation. 2) Up to a third in the variance of cancer incidence between populations can be attributed to a variation in diet, as highlighted in the recent World Cancer Research Fund report [6]. 3) Irritable bowel syndrome (IBS) affects 20% of the general population, many severely, and has associated co-morbidity with estimated annual losses of income and

productivity in the region of up to $30 billion (~ £20 billion)[7]. Gut flora of IBS subjects differ from controls, and may cause symptoms following consumption of certain foods, a mechanism for which is altered fermentation resulting in increased production of hydrogen gas[8]. 4) Most symbionts in humans can be divided broadly into two groups - Bacteroides and Firmicutes species. The balance between them is shifted in the obese (less bacteroides compared with lean individuals), suggesting that obesity has a microbial component[9]. An example of alteration in gut microbiota causing disease is *Clostridium difficile* infection. Prior administration of antibiotics is thought to shift the colonic bacterial composition by removing certain organisms which under normal circumstances restrain the *C. difficile* population. A further example is sepsis-related complications in liver cirrhosis resulting from bacterial translocation[10].

Anatomy of the colon in humans and animals

Constipation-predominant irritable bowel syndrome (IBS) and slow transit constipation are well recognised clinical entities amongst gastroenterologists. The unusual condition of proximal constipation in those with active distal colitis is recognised but poorly understood. Closer examination of plain radiographs of these patients suggests that the caecal contents have a "mushy" (or sponge-like) appearance probably as a result of gas bubbles entrapped within a loose network of solid material (fibre) (Figure 1). Video capsule endoscopy imaging has demonstrated gas bubbles in the vicinity of the ileo-caecal valve and the right colon. Our own experiments using faeces from two animal species (cow and horse) demonstrate the different radiographic appearances, reflecting perhaps differences in diet and colonic water and microbial content. Adding an equal quantity of sawdust (porous; allows air to be trapped between particles) to cow faeces creates virtually similar radiographic appearances between the two species (Figure 2). This supports our premise that the "mush" noted results from gas entrapped in solid. The digestive system in the calf resembles that of man[11] but as the animal grows, the ruminant

stomach occupies almost three quarters of the abdominal cavity. Thus fermentation in the ruminant which has a distinct gas liquid interface, takes place in the stomach whilst in man it occurs in the colon.

The need for a fermentation chamber

Faeces consists largely (80-90%) of water and its residue mainly of non-digested food stuffs, mostly non-starch polysaccharides (NSP). The latter is extracted by resident colonic bacteria and is the preferred energy source in colonocytes[12]. There are also bacteria which are key to fermentation, the chemical reaction through which gas is released. However, central to this process is the need for a suitable chamber, for which the right colon lends itself perfectly. It has an ordered inlet, i.e. the ileo-caecal valve. There is no equivalent valve at the outlet, i.e. the hepatic flexure, but we suggest that the local anatomy confers a valve-like action. The hepatic flexure is roughly right-angled or acutely angled, often "hitched" to the diaphragm (via the colo-phrenic ligament), and is partially rotated. The three strap muscles of the colon (teniae coli) align themselves at 120 degrees apart. Contraction (or relaxation) of the longitudinal strap muscles across the hepatic flexure exaggerates the "kink", thus creating a closed chamber in which fermentation can take place. The opposite action of the teniae conversely opens the chamber allowing its contents to progress through the colon.

The NSP and water content is significantly greater in the right colon (compared with the left) and acts as a multi-layered mesh to allow propagation of water, i.e. a sieve. This action in concert with resident colonic bacteria forms a 'biofilm', which may allow the formed gas bubbles to move slowly and occasionally become entrapped within faecal residue. The importance of these gas bubbles, we speculate, relates to the concept of quorum sensing[13]. These bacteria require some method of communication within this chamber to facilitate co-operation, division of labour and organisation, as well as survival. We believe these gases (VOCs) may fulfil this role.

We speculate that VOCs act as biotransmitters which influence colonic contraction and hence pressure; and as regulators of bacterial balance through quorum sensing. VOCs have a "chemical fingerprint"; suspected disorders can therefore be investigated by measuring change in the VOC profile.

Gut Ontology

Vertebrate gut development is extremely complex, requiring signalling between endoderm and ectoderm facilitating rotation on four major patterned axes[14]. As the gut matures, the distinct morphological and histological differences between foregut, midgut and hindgut become apparent. Whilst each of these compartments have a designated role, the hindgut, which has been traditionally associated with reabsorption of electrolytes and processing waste may, have a significantly more important role i.e. fermentation. It is plausible that the molecular mechanisms governing gut organogenesis in humans respond differentially allowing the ruminant to require a larger foregut (to aid fermentation), whilst in humans this role is in the hindgut.

Chemical Fingerprint – Implications for the future

A large proportion of colonic bacteria cannot be cultured; and new species identified through molecular fingerprinting are being added to the already-long list[15]. An alternative approach therefore is to study intestinal bacteria indirectly through their fermentation products, namely the VOCs, which serve as an acceptable surrogate.

To study colonic fermentation in health and disease requires a device which measures VOCs in faeces and produces an output showing the different components at their various molecular weights. An example of a VOC profile from *Bos Domesticus* (cow) is shown in Figure 3. The chemical identities of the VOCs can be identified by cross-reference to a database (Table 1). The VOCs profile reflects the metabolic activity of the bacteria that contribute to the fermentation profile and probably also indicates their relative proportions. Such proportions we suggest can be perturbed by changes in diet and other

environmental pressures. The resultant effect is a biomarker which is gaseous in nature – i.e. a novel non-invasive biomarker of colonic as well as metabolic disease.

Table 1: A selected list of Volatile organic compunds (VOCs) from cow faeces and corresponding retention times (related to chromatogram in Figure 3).

Retention Time	**Volatile organic compounds**
4.663	l-Alanine ethylamide, (S)-
4.773	Formic acid, ethenyl ester
5.04	Acetone
5.177	Dimethyl sulphide
5.379	2-Butanethiol, 2,3-dimethyl
5.64	2-Butanone
6.218	Butanal, 3-methy;-
7.347	1-Butanol, 3-methyl-
14.029	1-Hexanol, 2-ethyl-
21.349	Hexadecane
23.521	1,2-Bensenedicarboxylic acid, bis(2-methylpropyp) ester

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Figure 1: Plain radiograph of the abdomen showing faecal "mush" and presence of retained markers (arrow - day 7) indicating delayed colonic transit. In particular, note presence of 'bubbles' entrapped within contents.

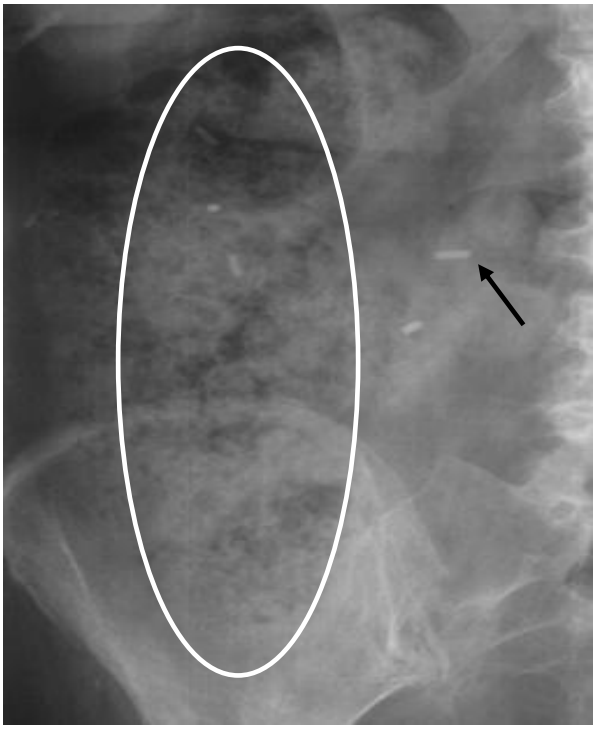


Figure 2: Radiographs showing A *Bos domesticus* (cow) faeces, B *Equine Cabulus* faeces and C a 50:50 mixture of cow faeces mixed with sawdust resulting in an appearance similar to B.

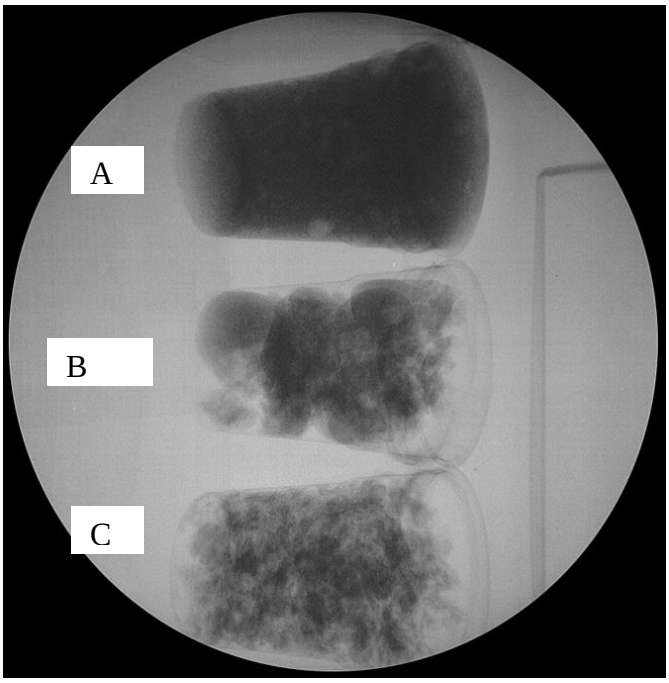
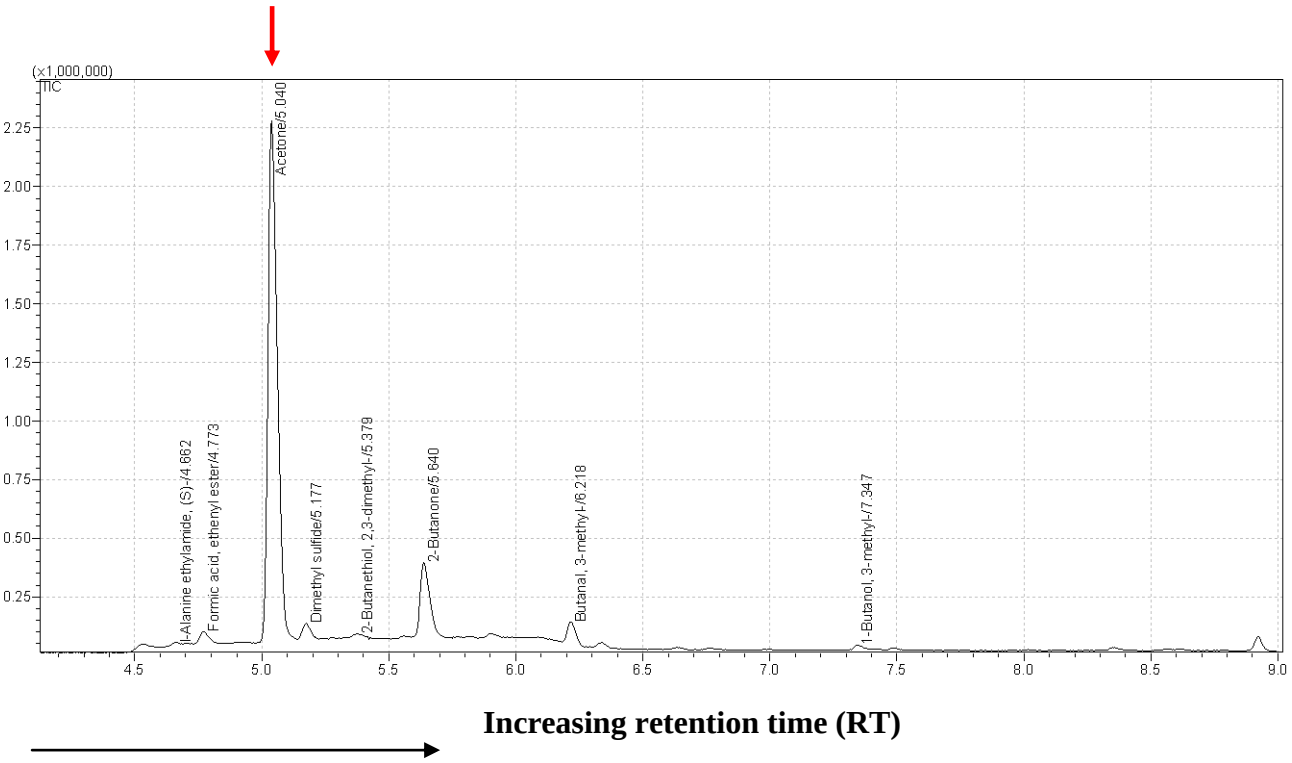


Figure 3: GC/MS chromatogram below shows a typical view of the VOC emitted from *Bos Domesticus* (cow) faeces. The tracing is similar to that of a complex electrocardiogram. The spectra seem to show several typical features particularly the presence of acetone. The presence of acetone (red arrow; RT = 5)in the volatile fingerprint is unexpected. As it is un-likely that the chemical is produced in the gut it is assumed that it is from the feed given to the cow. (PN short retention time indicates low molecular weight volatiles)

Quantity – as determined by ppm or area under the curve



Increasing retention time (RT)