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Leading article

Sulphate reducing bacteria and hydrogen metabolism in the human large intestine

One of the main functions of the large intestine is to salvage energy from dietary carbohydrate which has neither been digested nor absorbed in the small intestine. This occurs through a process known as fermentation in which anaerobic bacteria breakdown carbohydrate to short chain fatty acids (SCFA), which in turn provide energy for the host. The major carbohydrates available for fermentation are resistant starches, non-starch polysaccharides (dietary fibre), and a variety of unabsorbed sugars and oligosaccharides. Other substances can also be fermented, including dietary proteins and host derived substrates such as pancreatic enzymes, mucus, and sloughed epithelial cells.[1]

The fermentation of organic matter by gut micro-orgamisms is mediated by a number of interdependent reactions in which complex polymers are first broken down to their constituent monomers by hydrolytic enzymes synthesised by the bacteria. They are subsequently oxidised to SCFA, lactate, succinate, ethanol, and the gases H_2 and CO_2.[1] In some people, methane is also produced.[2] Fermentation is regulated by the amount of substrate available and its chemical composition, the substrate specificities and preferences of the bacterial species present and the metabolic pathways through which they metabolise the substrate.[3] Host factors such as transit time may also play a part.

Hydrogen is an important product of fermentation and is primarily formed by bacteria as a 'sink' for the disposal of reducing power (electrons) generated during oxidation of sugars and amino acids. In mammalian cells, oxygen is used as the terminal electron acceptor and reducing power is disposed of in the form of H_2O, this is not possible, however, in anaerobic metabolism. For example, some bacteria form ethanol, lactate, or succinate and do not evolve much H_2 because they dispose of reducing energy in these electron sink products.[4]

Hydrogen formation results principally from the oxidation of pyruvate, formate, or reduced pyridine nucleotides ($NADH^+$, $FADH^+$). Moreover, many clostridia generate H_2 from pyruvate via ferredoxin, whereas enterobacteria evolve the gas through cleavage of pyruvate by pyruvate formate lyase. The formate produced is then converted to CO_2 and H_2. The partial pressure of H_2 has no effect on its production from pyruvate, but accumulation of the gas inhibits formation from the oxidation of pyridine nucleotides. Since the regeneration of oxidised pyridine nucleotides through H_2 production enables increased substrate level phosphorylation to occur, an efficient H_2 removal may be a contributing factor in maintaining the fermentation balance in the colon. Hydrogen partial pressures in the colon are kept low by losses in breath and flatus[5–7] and by the activities of hydrogen utilising species such as methanogenic,[8] acetogenic,[9] and sulphate reducing bacteria.[10]

Between 30 and 50% of people in western countries harbour methanogenic bacteria in their colons.[2] These bacteria grow by reducing CO_2 and H_2 according to the following equation[11]:

$$4H_2+CO_2 \rightarrow CH_4+2H_2O$$

Thus, 4 mol of H_2 are converted to 1 mol of CH_4. Methane production is an effective and safe pathway for H_2 disposal and occurs in most animals. In people who do not excrete CH_4, other routes for microbial H_2 disposal frequently exist. An important alternative is the reduction of sulphate by sulphate reducing bacteria.[12] We have shown that sulphate reducing bacteria occur in large numbers (ca 10^8–10^{10} (g dry weight gut contents)^1) in non-methanogenic but not in methanogenic individuals.[13] Sulphate reducing bacteria utilise H_2 according to the following equation[11]:

$$4H_2+SO_4^{2-}+H^+ \rightarrow HS^-+4H_2O$$

As in methanogenesis, 4 mol of H_2 are consumed in the formation of 1 mol of product. Unlike methanogenesis, however, the product is not a harmless gut, but is highly toxic hydrogen sulphide, an agent that is potentially damaging to the colonic epithelium.[13] People who harbour sulphate reducing bacteria in their large bowel have higher levels of sulphide in their faeces than methanogenic subjects.[12] The main substrates for colonic sulphate reducing bacteria are fermentation products formed by other bacteria, such as acetate, propionate, lactate, butyrate, succinate, ethanol, pyruvate, some amino acids, and H_2/CO_2.[14] Species that can utilise long chain fatty acids and alcohols (up to C6) have also been isolated by chemostat enrichment.[15] These strict anaerobes are the final link in the food chain that develops in the gut ecosystem and use sulphate as a terminal electron acceptor during oxidative reactions.[16] The predominant sulphate reducing bacteria found in the gut belong to five genera, namely *Desulfovibrio*, *Desulfobacter*, *Desulfomonas*, *Desulfobulbus* and *Desulfotomaculum*.[17] Species that use molecular H_2 as an electron donor in the gut belong to the genera *Desulfovibrio* and *Desulfobulbus*. Desulfovibrios account for approximately 66% of all colonic sulphate reducing bacteria, while *Desulfobulbus* spp. constitute about 16%.[17]

Fermentation experiments in our laboratory have shown that colonic sulphate reducing bacteria outcompete methanogenic bacteria for H_2.[18] More recently, however, Strocchi *et al*[19] have postulated that methanogens were able to competitively displace other H_2 consuming bacteria in mixed faecal slurries. In this study, sulphate reducing bacteria must have been absent, or their activities limited in the non-methanogenic samples tested, because it is highly unlikely that methanogenic bacteria will displace sulphate reducing bacteria for this mutual growth substrate given an adequate supply of electron acceptor (sulphate). The physiological explanation for this resides in the greater affinity of sulphate reducing bacteria for H_2 compared with methanogenic bacteria (Ks of *Desulfovibrio vulgaris*, 1 $\mu mol.l^{-1}$; Ks of *Methanobrevibacter smithii*, 6 $\mu mol.l^{-1}$).[20] Moreover, the oxidation of H_2 by sulphate reducing bacteria is thermodynamically more favourable ($\Delta G_0' = -152{\cdot}2$ kJ per mol) than by methanogenic bacteria ($\Delta G_0' = -131$ kJ per mol).[11] The outcome of competition is largely dependent on sulphate availability, with sulphate reducing bacteria outcompeting methanogenic bacteria for the mutual growth substrate H_2

in the presence of sulphate.[21-31] Strocchi *et al*[19] did not enumerate viable sulphate reducing bacteria in their study, and the addition of 20 mM sulphate to non-methanogenic faecal slurries made little difference to sulphide production, indicating an absence of viable sulphate reducers. In contrast, we have found that in two geographically and nutritionally diverse populations, viable sulphate reducing bacteria were present and active in all non-methanogenic samples tested.[14]

Thus, if sufficient sulphate is available in vivo, our belief is that sulphate reducing bacteria will be responsible for most of the H_2 utilised by the gut microflora. When sulphate cavailability is limited, methanogenic bacteria may then predominate. Other lines of evidence support this hypothesis. For example, if sulphate is added to the diet of methanogenic individuals, competitive displacement of methanogenic bacteria occurs and hitherto undetectable sulphate reducing bacteria begin to appear in faeces within a few days.[32] This, effect is observed only in about 50% of methanogenic individuals, however, suggesting that sulphate reducing bacteria and methanogenic bacteria coexist in some, but not all, people. Furthermore, in whole body calorimetry studies when total H_2 and CH_4 excretion were measured in healthy subjects eating test meals of various fermentable carbohydrates, the amount of excreted H_2 that could be accounted for was much less in non-methanogenic subjects. For example, in response to a 15 g dose of lactulose, methanogenic subjects excreted mean (SEM) 1150 (212) ml hydrogen equivalents, while non-methanogenic volunteers excreted only 327 (88) ml.[33] These data support the existence of alternative pathways for H_2 disposal in man.

For sulphate reducing bacteria to outcompete methanogenic bacteria for H_2 in the colon, there must be an adequate supply of sulphate. Studies of sulphate absorption using ileostomists have indicated that concentrations of this anion can vary considerably in diet and that amounts reaching the colon may range from 2 to 9 mmol.day^{-1}.[34] Other potential sources exist, including highly sulphated polysaccharides such as chondroitin sulphate.[35 36] Colonic mucins are also highly sulphated, more so in fact than mucins produced elsewhere in the gastrointestinal tract.[37 38] There are many species of bacteria in the colon including clostridia, bifidobacteria, and bacteria of the *Bacteroides fragilis* group that are able to degrade these substances and thereby release free sulphate[39-45] which would then become available for utilisation by sulphate reducing bacteria. If this is indeed the case, then the amount of sulphated glycoprotein produced by the host, its degree of sulphation, and the activities of the hydrolytic bacteria involved in releasing free sulphate from these polymers, would all influence sulphate reducing bacterial growth. Because mucin structure and production varies between individuals, there may possibly be a genetic predisposition to the acquisition of sulphate reducing bacteria.

Another pathway of H_2 disposal is by the activities of acetogenic bacteria, which also grow in the colon. Species that reduce CO_2 with H_2 do so according to the following equation[11]:

$$4H_2+2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$$

Acetate production from CO_2 occurs in the rat caecum[46] and in the gut of termites.[47] Significant acetate production as a result of H_2 dependent CO_2 reduction has also been reported in human faeces in the absence of methanogenesis.[48] In terms of energy, however, H_2/CO_2 acetogenesis, with a free energy change of -95 kJ per mol,[11] is considerably less efficient than either dissimilatory sulphate reduction or methanogenesis, and in most anaerobic ecosystems, hydrogen metabolism is dominated by one or other of these processes.[49 50] However, pH can influence the outcome of competition, with acidic conditions selecting for acetogenesis,[12] and it is possible therefore that the luminal pH in the colon is important in this respect. Nevertheless, it is unlikely that homoacetogenesis is a major route of disposal of H_2 in the human colon since molar ratios of acetate, propionate, and butyrate are similar in methanogenic and non-methanogenic people.[51] Comparison of SCFA concentrations in the gut of known acetogenic animals, such as the termite, with man, shows that in the insect acetate accounts for 94% of the total compared with only 57% in the human caecum.

What are the clinical implications of all this? Effective H_2 disposal during fermentation is essential for normal large bowel function. In man, the principal routes are via methanogenesis or dissimilatory sulphate reduction. Methanogenesis is a safe and effective disposal route for H_2. Methane is non-toxic and allows a large volume of H_2 to be removed, thereby potentially reducing any gas related symptoms. In the absence of either methanogenic bacteria or sulphate reducing bacteria in the colon, H_2 accumulates in excessive quantities and this may be an important factor in pneumatosis cystoides intestinalis.[52] Other ways of disposing of H_2 include acetogenesis and dissimilatory nitrate reduction, for which H_2 is the preferred electron donor. Acetogenesis is probably the most favourable H_2 disposal method, since this makes additional energy available to the host from fermentation through acetate absorption. However, the evidence for significant homoacetogenesis in man is still inconclusive.

Sulphate reduction is probably the least desirable route. The main product, sulphide, is toxic to cells and impairs cellular metabolism through inhibition of cytochrome oxidase.[53 54] Sulphide may also destroy the disulphide bridges in mucus, in turn leading to a breakdown of the protective layer of the epithelium. Moreover, other sulphydryl compounds have been implicated in colonic disease since Roediger *et al*[55 56] have shown that mercaptoacetate and mercaptobutyrate affect fatty acid metabolism in colonocytes in a manner that is characteristic of the defect found in ulcerative colitis. We have shown that ulcerative colitis patients have a very high carriage rate of sulphate reducing bacteria and raised sulphide values in faeces.[57 58]

Thus the removal of H_2 in the large gut may follow a number of pathways. It is clear from studies in man that either methanogenesis or dissimilatory sulphate reduction predominate in some populations. Because of the known toxicity of sulphide in the gut we suggest that the role of sulphate reducing bacteria in the pathogenesis of colonic disease warrants further investigation.

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