

## *Leading article*

# Fibre, fermentation, flora, and flatus

Research into flatogenesis has undergone several, apparently unrelated, phases. Early studies of the volume of gas in the gastrointestinal tract<sup>1,2</sup> were prompted by the abdominal pain and discomfort experienced by sailors and airmen who had been subjected to explosive decompression. The second phase was aided by the realisation that human gas production could pose an explosion hazard during longterm space flight.<sup>3</sup> Since that time, there has been little attempt to quantitate gas production in man although there have been many studies on concentrations of H<sub>2</sub> and CH<sub>4</sub> in end expired breath. There have been remarkable advances during the last 15 years in our understanding of the nature of non-absorbed sugars and dietary fibre and of their fermentation in the large intestine. It is now possible to construct a clearer picture of the overall stoichiometry of colonic fermentation and gas production in man.

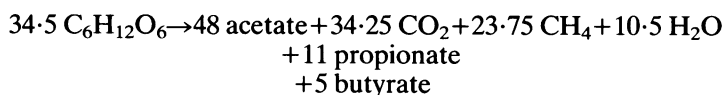
The link between large intestine fermentation of non-absorbed carbohydrate and gas production has been exploited in numerous breath tests for malabsorption because a portion of the gas produced (H<sub>2</sub> and CH<sub>4</sub>) is absorbed from the lumen of the large intestine into the blood stream and excreted in the lungs. As much as 13% of H<sub>2</sub> gas may be lost through this route.<sup>4</sup> Whilst H<sub>2</sub> appears to be the major unique gas produced by intestinal fermentation, a proportion of individuals also excrete appreciable quantities of CH<sub>4</sub>.<sup>5–14</sup> In patients with unresected colon cancer the proportion of methane-producers is high (86–92%), falling to normal levels (43–47%) after resection.<sup>13</sup> Methanogenic bacteria are also present in large quantities in patients with diverticulosis.<sup>15</sup> In contrast with these data, a recent study of South African populations has shown that although there was a wide variation in the proportion of methane producers in different ethnic groups, this did not relate to the relative risk of developing colon cancer.<sup>16</sup> The picture is confusing and the report of Weaver and colleagues,<sup>17</sup> in this issue of *Gut* is therefore timely. In this study, an attempt has been made to determine the stoichiometry of fermentation of defined substrates by incubation of faecal samples from a H<sub>2</sub> and a CH<sub>4</sub> producer. It addresses the essential questions of 'How much', 'Why?', 'Where?', 'What?', and 'Why not?'.

### **How much?**

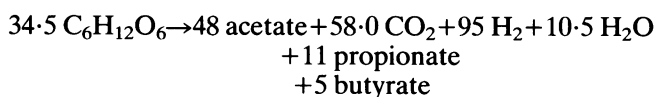
Most studies have measured only the small proportion of H<sub>2</sub> and CH<sub>4</sub> which is excreted in the breath. Because H<sub>2</sub> and CH<sub>4</sub> are expressed as concentrations, it is not possible to calculate total output unless respiratory volumes are known. End expiratory gas measurements are straightforward in patients or volunteers, but rectal intubation for measurement of flatus volumes is not. A non-invasive alternative to rectal intubation is suggested

by the report of Garlick and colleagues,<sup>18</sup> in which total <sup>14</sup>C<sub>2</sub>O<sub>2</sub> output was measured during infusion with <sup>14</sup>C-labelled amino acids. Each subject was housed in a ventilated, disposable oxygen tent and measurements of gas flow and gas composition made with simple equipment. This technique may be useful in quantitating intestinal gas production in man. There is evidence that humans are considerably less flatogenic than theory would suggest and the reason for this may relate to the health of the large intestine.

A reason for believing that man passes less wind than he should is suggested by the elegant study of Weaver and colleagues.<sup>17</sup> In an earlier review of the subject,<sup>19</sup> the stoichiometry of colonic fermentation in man was proposed to be:



Alternatively, if no CH<sub>4</sub> were produced by CO<sub>2</sub> reduction, the equation could be rewritten:



In Weaver's<sup>17</sup> study faecal suspensions from both subjects fermented glucose and corn starch to produce VFA's and gas, although the relative proportions of each product varied. The VFA, CH<sub>4</sub>, and H<sub>2</sub> yields per glucose molecule were considerably lower than would be expected by either equation.

A second reason is suggested by considering the amounts of carbohydrate which are fermented in the large intestine. It is thought that an average value should be approximately 70 g, calculated on the basis of the amount required to produce the observed bacterial mass of human faeces.<sup>20</sup> In other words, to the quantity of fermentable non-starch polysaccharide normally ingested<sup>21, 22</sup> should be added the significant quantities of starch which may be present in the diet in a form which resists small intestinal amylolysis and brush border assimilation of constituent glucose moieties.<sup>23-25</sup> Fermentation of 70 g of carbohydrate would produce, by either scheme described above, either 6 litres/d of CH<sub>4</sub> or a truly Zeppelinogenic 24 litres/d of H<sub>2</sub>. Flatus volume, however is rarely more than 1 litre/day.<sup>3, 4, 26</sup>

Where, therefore does all of the gas go and what may be the significance of submaximal flatogenesis? The pattern of carbohydrate fermentation by anaerobic bacteria in the large intestine may explain part of this difference.

### Why?

Metabolic events in the lumen of the large bowel occur under anaerobic conditions such that complete oxidative microbial metabolism of carbohydrate by intestinal flora cannot occur through the Krebs's Cycle. Limited oxidation of sugars and their intermediary metabolites can only proceed if reduced equivalents are removed by a 'metabolic sink'. H<sub>2</sub> and CH<sub>4</sub> production therefore provides a simple means of removing reduced equivalents, in gaseous form. The energy yield of carbohydrate to the bacteria is rather low and further oxidative metabolism of the bulk of

volatile fatty acids (VFA's) produced has to occur within the colonocyte, or in other tissues after uptake and transmucosal transport. An elegant summary of the metabolic pathways by which organic acids, and in particular the VFA's, are produced by different classes of colonic bacteria, is given elsewhere.<sup>19 20 27</sup> The major feature of these pathways is that hexose sugars are cleaved to form pyruvate, through the Emden-Meyerhoff Pathway. Two pathways are then available for disposal of pyruvate. First, it may be reductively converted to propionate (through succinate). The second pathway converts pyruvate to acetyl-CoA which may then either be hydrolysed (to release acetate), or reduced to form butyrate. Conversion of pyruvate to acetyl-CoA produces reducing equivalents as H<sub>2</sub>, whereas formation of propionate and butyrate consume H<sub>2</sub>. If, however, anaerobic degradation of sugars were allowed to proceed to completion, the end products would be CO<sub>2</sub> and CH<sub>4</sub>.

It is therefore clear that the extent of methanogenesis will determine the pattern of VFA's formed in the intestine. The major species responsible for methane production in man is *Methanobrevibacter smithii*.<sup>6 7</sup> CH<sub>4</sub> is formed by this organism from CO<sub>2</sub> and H<sub>2</sub> produced by other bacteria. It seems that in man (as in the ruminant), the time required by acetate using methanogens to oxidise all fatty acids and H<sub>2</sub> to CO<sub>2</sub> and CH<sub>4</sub> is longer than large bowel transit time.<sup>20</sup> Production of methane from CO<sub>2</sub> and hydrogen results in decreased gas volumes, because one volume of methane is produced from four volumes of H<sub>2</sub>. This may explain in part, submaximal flatogenesis in man.

Different bacterial species are responsible for H<sub>2</sub> and CH<sub>4</sub> generation and there are variations in the dynamics of their production in man. For the readily fermentable carbohydrate, lactose, one half of H<sub>2</sub> production occurs in the caecum and right colon, compared to less than 10% of methane production.<sup>4</sup> Ingestion of lactulose and breath H<sub>2</sub> also correlate well<sup>28</sup> and the evidence suggests that malabsorption of a test carbohydrate should be 'calibrated' by normalising the breath H<sub>2</sub> response to that of a known amount of lactulose.<sup>29</sup> In methane producers, however, the breath CH<sub>4</sub> response to oral lactulose is idiosyncratic<sup>12</sup> and may relate to the rapid fermentation of this substrate in the caecum and right colon such that little is available for further fermentation at more distal, methanogenic sites. Despite the usefulness of breath H<sub>2</sub> tests, there are several caveats. First, preexisting treatment (antibiotics, enema or colonoscopy) markedly affects H<sub>2</sub> production.<sup>30 31</sup> More subtly, mood dramatically alters breath H<sub>2</sub> output, which is increased during periods of acute<sup>3 32</sup> or chronic<sup>3</sup> tension or when gas measurements are made in public places.<sup>32</sup> This may be caused either by a sudden increase in the amount of fermentable substrate reaching the caecum and right colon (during periods of stress), or to retention of gas (and an increase in its excretion in breath). It is therefore clear that care should be exercised in designing diagnostic H<sub>2</sub> breath tests and that attention should be paid to the comfort and privacy of the subjects.

Interestingly, methane production is unresponsive to mood,<sup>32</sup> or pre-existing treatment,<sup>31</sup> again suggesting that H<sub>2</sub> and CH<sub>4</sub> production are partially separated by anatomy and microbiology in the human large intestine and may be independently altered by transit phenomena. The nature of the non-absorbed carbohydrate may therefore be crucial to the quantities of gas produced.

## Where?

The flatogenic potential of all non-absorbed carbohydrates should be the same, if all saccharide bonds are available for hydrolysis and constituent monosaccharides liberated for subsequent bacterial fermentation. This is clearly not the case, however, as shown by the variation in fermentation of non-starch polysaccharides in man<sup>33</sup> and in the pig.<sup>34</sup> Variability seems to be determined not only by the type of saccharide linkage involved, but also by the physicochemical characteristics of the non-absorbed polysaccharide – such as the ‘resistant starches’.<sup>35–37</sup> For non-starch polysaccharides, availability relates more to physical, rather than chemical factors.<sup>38</sup> The access of bacterial cell wall polysaccharidases<sup>27</sup> to bonds buried deep within highly ordered, relatively dehydrated polysaccharide structures may be limited. Decreased fibre particle size,<sup>38–40</sup> increased hydration (due to removal of lignin during digestion<sup>41</sup>), increased fibre porosity and solubility and decreased viscosity<sup>42</sup> all tend therefore, to increase the fermentability of non-absorbed carbohydrates. Fermentation of soluble, non-absorbed sugars (Lactulose, Lactitol, Palmitit, Isomalt) in the caecum and right colon is limited by none of these factors and they all express their full fermentative and flatogenic potential when administered orally.<sup>43–46</sup>

The possibility therefore exists that hydrolysis and fermentation of different non-absorbed polysaccharides may occur at different sites in the large intestine. Levitt and Ingelfinger<sup>4</sup> observed that methane production occurred primarily in the left colon. Dietary fibre which is poorly hydrolysed and fermented in the right colon may provide a good supply for H<sub>2</sub> and CH<sub>4</sub> production in the left colon. At present this concept is speculative but would be supported by the observation that not only is CH<sub>4</sub> production by methanogenic bacteria pH dependent<sup>47</sup> but that these strains have increased growth rates at slightly alkaline pH.<sup>48</sup> Thus their growth would be most favoured by conditions in the left colon. The decrease in large bowel transit time produced by a high fibre diet may, however, lower bacterial contact time and CH<sub>4</sub> production. This may be inferred from the observation that large bowel transit time and faecal pH correlate significantly with breath methane levels<sup>49</sup> although in the recent study of Segal *et al.*<sup>16</sup> this was not the apparent cause of high levels of methane production in rural black South Africans who had rapid transit consequent on high fibre consumption.

## What?

The proportions of gases produced during colonic fermentation may provide a clue to the pattern of VFA production. Weaver and colleagues<sup>17</sup> in this issue of *Gut* provide some data on this point. Volatile fatty acids and gas production were measured, using glucose or starch as the substrate. In the hydrogen producer, glucose and starch disappearance were similar, indicating that hydrolysis of starch was not rate limiting for fermentation. In contrast, starch appeared to be less well fermented than glucose, in the methane producer. The pattern of VFA's also differed. Methanogenesis was accompanied by less propionate and more acetate production. Similar observations have been made in ruminants when ciliate protozoa were established in the rumen: a significant negative correlation between propionate and CH<sub>4</sub> production, was reported.<sup>50</sup> These changes may have an

impact on the health of the large bowel. It has been suggested<sup>51</sup> that VFA's may have a protective role in relation to colon cancer. Butyrate is a major substrate for colonocyte energy metabolism<sup>52</sup> and has cytostatic effects on colorectal cell lines.<sup>51</sup> It has recently been shown that VFA's stimulation of crypt cell production rate in the small and large intestine of rats occurs *in vivo* (butyrate > propionate > acetate), but this may be mediated systemically, as all VFA's inhibit epithelial cell proliferation *in vitro*.<sup>53</sup> Factors which reduce VFA production may therefore be of importance. Methane production represents a 'sink' into which bacteria may dump reducing equivalent, thus releasing the coenzymes NAD, NADP, and FAD for further bacterial oxidative metabolism of VFA's. In ruminants and to a lesser extent in pigs, methanogenesis represents a loss of VFA's to the host, which would otherwise be absorbed for further metabolism.<sup>50,54</sup>

The possibility therefore exists that excess methane production may be associated with two, possibly deleterious, phenomena. First, it may tend to decrease the supply of readily metabolised substrate (VFA's) to the colon. It may also alter the production of specific VFA's with growth regulatory properties within the large bowel lumen. The experimental evidence for trophic effects of VFA's on small and large bowel function during enteral nutrition are reviewed elsewhere.<sup>55</sup> This is clearly an important area and warrants further study.

### Why not?

The relationship between H<sub>2</sub> and CH<sub>4</sub> production, the type of fermentable non-absorbed polysaccharide and sites of fermentation is not clearly known. Perfusion and representative sampling of these sites may not be possible without disturbing the balance of microflora within these regions. Attention has therefore been given to incubation systems which mimic the anatomy of the large bowel by use of sequential fermentation chambers.<sup>56,57</sup> This strategy has been used to study rumen fermentation and the digestion of dietary proteins in the stomach and small intestine.<sup>58</sup> Site and time related aspects of carbohydrate assimilation may thus be studied in a continuous culture system which is not dissimilar to the human colon. Using this approach, the impact of sulphate reducing bacteria (SRB) on gas and VFA production was determined.<sup>57</sup> The three chambers were pH controlled to simulate the pH gradient between the caecum and left colon and after initial inoculation with a faecal slurry the system was allowed to reach equilibrium. During infusion of porcine gastric mucin (containing sulphated mucopolysaccharides) CH<sub>4</sub> and H<sub>2</sub> production were completely suppressed in the two distal chambers operated at higher pH. This was accompanied by sulphate reduction (by H<sub>2</sub>) and generation of sulphide, catalysed by increasing numbers of SRB. After the mucin infusion ceased, the system returned to its steady-state. In separate experiments with incubated faecal slurries from methane producers and non-methane producers, significant quantities of CH<sub>4</sub> were only produced in the absence of sulphate reduction. Mixtures of slurries from both sources showed the ability of SRB to outcompete methanogenic bacteria (MB) for H<sub>2</sub>.<sup>59</sup> Addition of nitrate,<sup>59</sup> which can be reduced to nitrite by other classes of faecal anaerobes<sup>60</sup> variably suppressed CH<sub>4</sub> and H<sub>2</sub>S production in both types of slurries. The suggestion that establishment of either MB or SRB colonies (but not both)



occurs in the human colon is supported by the report that SRB in faecal samples from British and South African populations correlated inversely with breath CH<sub>4</sub> production.<sup>61</sup> The supply of sulphate containing mucin to the colon may be the major determinant of SRB colonisation.<sup>59</sup> Whatever the reason for establishment of SRB in the human colon, it will result in diversion of H<sub>2</sub>, away from CH<sub>4</sub> (and thus flatogenesis), and towards non-volatile S<sup>2-</sup> production.

## Conclusion

Research into fibre, fermentation, flora, and flatus has reached an exciting point. There is now a clearer understanding of the stoichiometry of large intestinal fermentation, the ways in which the colonic microflora utilise different types of non-absorbed polysaccharides and also the effects of VFA's on growth of normal and transformed colocytes. What is particularly interesting is the extent to which man is an underperformer in the flatogenic stakes. Secondary utilisation of H<sub>2</sub> by MB, SRB, and nitrate reducing bacteria may account for all of this difference, but quantitative data are lacking. The author would suggest that interest in whole body flatometry be revived. It would not be unreasonable to determine the overall stoichiometry of fermentation and H<sub>2</sub> disposal from the extent to which H<sub>2</sub> and CH<sub>4</sub> production fall short of theoretical yields for a particular dietary fibre, whose digestibility can be defined with the methods of analysis which are now available.

The observation that methanogenic or sulphate reducing bacteria are established in the colon, to the exclusion of the other, is particularly fascinating. Does this represent an underlying difference in the intrinsic sulphation pattern of epithelial mucopolysaccharides which may ultimately be fermented in the large intestine? It is hard not to consider this a good kite to fly in future research.

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