

LETTERS TO THE EDITOR

Methanogenesis in the human large intestine

EDITOR,—Methanogenesis is important in many anaerobic microbial environments. The human colon is such an environment. It is therefore a matter of curiosity that only some humans consistently produce significant amounts of methane. *Gut* published a paper concerning the apparent regulation of colonic methanogens by sulphate from the human diet (*Gut* 1992; 33: 1234–8). Sulphate is a substrate for oxidative metabolism by sulphate reducing bacteria. A crucial argument of the authors of this paper is that sulphate reducing bacteria outcompete gut methanogens for hydrogen. They base this on their own *in vitro* work,¹ even though there are generally only small amounts of sulphate in faeces from humans on a high sulphate diet.²

Other investigators have reported the complete opposite. That is, human methanogens outcompete sulphate reducing bacteria in mixed faecal cultures, even with sulphate added^{3,4}; that human methanogenic faeces consume hydrogen far more rapidly than non-methanogenic faeces;⁵ and that faecal concentrations of sulphate and sulphide, the substrate and end products of sulphate reducing bacteria, are not even appreciably different between human methanogenic and non-methanogenic faeces.^{2,5}

At least two of the three authors of the paper published in *Gut* knew of this other work as long ago as mid 1990. It is, therefore, disturbing and a little disappointing that this research work^{2,5} was not referenced in their paper.

The control of human methanogenesis is not because of a competition between methanogens and sulphate reducing bacteria, so what does regulate human methanogenesis? There is good evidence that bile^{6,7} and faecal stirring⁸ inhibit hydrogen consumption by human methanogens. These are two of several possible factors, which could explain in a large part the epidemiology of human methanogenesis, including its rarity in patients with Crohn's disease of the terminal ileum. Ingested sulphate might act by augmenting gut motility and bile losses into the colon.⁹

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- Gibson GR, Cummings JH, Macfarlane GT. Competition for hydrogen between sulphate-reducing bacteria and methanogenic bacteria from the human large intestine. *J Appl Bacteriol* 1988; 65: 103–11.
- Florin THJ, Neale G, Gibson GR, Christl SU, Cummings JH. The metabolism of dietary sulphate: absorption and excretion in man. *Gut* 1991; 32: 766–73.
- Florin THJ. Hydrogen sulphide and total acid-volatile sulphide in faeces, determined with a direct spectrophotometric method. *Clin Chim Acta* 1991; 196: 127–34.
- Strocchi A, Furne JK, Ellis JC, Levitt MD. Competition for hydrogen by faecal bacteria: evidence for the predominance of methane producing bacteria. *Gut* 1991; 32: 1498–501.
- Lemann F, Pochart P, Pellier P, Flourie B, Rambaud JC. Does sulphate availability in human colonic content control methanogenesis in man? *Gastroenterology* 1990; 98: A251.
- Florin THJ, Jabbar IA. Factors controlling human bowel metabolism: the effect of bile on methanogenesis. *J Gastroenterol Hepatol* 1992; 7: A21.

- Xu B, Xi M, Wang Y, Lu D, Xiao S. Demonstration of the presence of *Methanobrevibacter* in a colon cancer chinese patient. *Acta Microbiol Sin* 1990; 30: 400–2.
- Strocchi A, Levitt MD. Factors affecting hydrogen production and consumption by human fecal flora. *J Clin Invest* 1992; 89: 1304–11.
- Harvey RF, Read AE. Saline purgatives act by releasing cholecystokinin. *Lancet* 1973; ii: 185–7.

Reply

EDITOR,—The study of hydrogen metabolism in the human large intestine is providing new insights into colonic function and it is now being recognised that this may have implications for a number of diseases.^{1–4} The control of methane production, which is one of a variety of ways for hydrogen disposal in man, has hitherto been incompletely understood. There is now substantial evidence, however, that competition for the growth substrate H₂ exists between methanogenic bacteria and sulphate reducing bacteria in some human populations.^{5,8}

Since 1988⁵ we have extended our studies of normal human faecal flora from the original 40 subjects to 127 and have found that 59 (46%) are methanogenic and 68 (53%) sulphate reducing. In 16 of the methanogenic subjects, low numbers of sulphate reducing bacteria were found and significant sulphate reducing activity occurred when methanogenesis was inhibited. *In vitro*, we have shown that competitive interactions occur between methanogenic bacteria and sulphate reducing bacteria, and that in the presence of sulphate from either organic (mucin) or inorganic sources, sulphate reducing bacteria are able to outcompete methanogenic bacteria for hydrogen.^{7,8} In the paper,⁹ sulphate feeding inhibited methanogenesis in half of the subjects when sulphate reducing bacteria were provided with an adequate amount of electron acceptor (sulphate). Such interactions between methanogenic bacteria and sulphate reducing bacteria also occur in marine sediments.¹⁰ Methanogenic bacteria will always be outcompeted by sulphate reducing bacteria if sulphate is available unless hydrogen is present in great excess, the reason being that K_s for H₂ uptake favours sulphate reducing bacteria (1 μmol/l for *Desulfovibrio vulgaris*) at the expense of methanogenic bacteria (6 μmol/l for *Methanobrevibacter smithii*).¹¹ Also, the oxidation of H₂ by sulphate reducing bacteria is thermodynamically more favourable ($\Delta G_0' = -152.2$ kJ/mol) than by methanogenic bacteria ($\Delta G_0' = -131$ kJ/mol).¹²

In this context, Dr Florin raises the interesting question of why there is no significant sulphate or sulphide in the faeces of methanogenic subjects.^{13,14} As he will recall from the three years he spent with us in Cambridge, there are several possible explanations for this. The most important is that many species of facultatively anaerobic bacteria such as *Escherichia coli* can use sulphate as their sole source of elemental sulphur. In so doing, they reduce sulphate, a process known as assimilatory sulphate reduction.¹⁵ By contrast, sulphate reducing bacteria conduct dissimilatory sulphate reduction in which sulphate acts as an electron acceptor during the breakdown (dissimilation) of organic matter. Both pathways occur in the colon and consume sulphate. Sulphide (SH⁻) is of course rapidly absorbed and oxidised by the colonic mucosa.

Dr Florin suggests that other investigators have reported 'the complete opposite to be the case' namely that methanogenic bacteria outcompete sulphate reducing bacteria. His refer-

ences do not support this argument. His own paper¹⁶ which is essentially about measuring sulphide, contains no competition studies between methanogenic bacteria and sulphate reducing bacteria, or even data from mixed faecal slurries. The other paper¹⁴ does include competition studies but no evidence that the non-methanogenic faeces contained viable sulphate reducing bacteria. No study, where sulphate was available in appreciable amounts, has shown that methanogenic bacteria outcompete sulphate reducing bacteria. It is worth noting that where an apparent absence of sulphate reducing bacteria is seen it is necessary to enumerate these bacteria after sulphate feeding, not just before, as such feeding may lead to growth of sulphate reducing bacteria which had not been detected before.⁹

So what of Dr Florin's suggestion that other factors control methanogenesis? We are happy to accept the findings of Levitt's group¹⁷ that faecal stirring may be important. Stirring could increase the availability of hydrogen to methanogenic bacteria and thus allow bacteria to gain some advantage over organisms which might normally outcompete them in a more restricted environment. We note Strocchi and Levitt's comments about there being no quantitative data on stirring of colonic contents (*in vivo*).

We are not convinced of the role of bile as a controlling factor. Dr Florin may have misinterpreted his own experiments, which he reports in abstract form.¹⁸ Every healthy colon contains bile acids, with which colonic bacteria coexist quite effectively. Miller and Wolin^{19,20} have shown that methanogenic bacteria from the colon are not inhibited in their function by bile acids. These studies were conducted with *Methanobrevibacter smithii*¹⁹ and *Methanosphaera stadtmaniae*,²⁰ the principal colonic methanogens. Of course, if you have enough bile acid then some inhibitory effects may be possible. Bile acid concentrations in ileal effluent, however, are less than 1 mM^{21,22} while in the aqueous phase of stool they are even lower.^{23,24} Dr Florin's studies, which actually failed to show an effect of bile acids on methanogenesis at concentrations of 0.05% (around 1 mM) were at concentrations of 0.1–1.0% bile acids (2.5–25.0 mM), which is unphysiological.

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- Gibson GR, Macfarlane GT, Cummings JH. Sulphate reducing bacteria and hydrogen metabolism in the human large intestine. *Gut* (*in press*).
- Christl SU, Gibson GR, Murgatroyd PR, Schepach W, Cummings JH. Impaired hydrogen metabolism in pneumatosis cystoides intestinalis. *Gastroenterology* (*in press*).
- Gibson GR, Cummings JH, Macfarlane GT, Allison C, Segal I, Vorster HH, *et al*. Alternative pathways for hydrogen disposal during fermentation in the human colon. *Gut* 1990; 31: 679–83.
- Gibson GR, Cummings JH, Macfarlane GT. Growth and activities of sulphate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. *FEMS Microbiol Ecol* 1991; 86: 103–12.
- Gibson GR, Macfarlane GT, Cummings JH. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. *J Appl Bacteriol* 1988; 65: 103–11.
- Lemann F, Pochart P, Pellier P, Flourie B, Rambaud JC. Does sulphate availability in colonic content control methanogenesis in man? *Gastroenterology* 1990; 98: A186.
- Gibson GR, Cummings JH, Macfarlane GT. Competition for hydrogen between sulphate-reducing bacteria and methanogenic bacteria