Alternative pathways for hydrogen disposal during fermentation in the human colon

G R Gibson, J H Cummings, G T Macfarlane, C Allison, I Segal, H H Vorster, A R P Walker

Abstract

Hydrogen gas, which is produced during fermentation in the human colon, is either excreted in breath or metabolised by gut bacteria through a variety of pathways. These may include methanogenesis, dissimilatory sulphate reduction, and acetogenesis. To determine which of these routes predominates in the large intestine, stools were taken from 30 healthy subjects and incubated as 5% (w/v) slurries with Lintner's starch. In 23 of 30 subjects, methane production was the main method of hydrogen disposal. In the remaining seven, high rates of sulphate reduction were recorded together with raised production of H_2S. All samples showed relatively low rates of hydrogen evolution and of acetate formation from CO_2 and H_2. Sulphate reduction and methanogenesis seem to be mutually exclusive in the colon and this is probably linked to sulphate availability. Sulphate reduction, methanogenesis, and acetogenesis were strongly influenced by pH. Sulphate reduction was optimal at alkaline pH values whereas methane production was maximal at a neutral pH and acetogenesis favoured acidic conditions. Faecal H_2S values were related to carriage of sulphate reducing bacteria. These data show that a number of competing pathways for hydrogen disposal are possible in the large gut and that a variety of factors such as colonic pH and sulphate availability can determine which of these mechanisms predominates.

MRC Dunn Clinical Nutrition Centre, Cambridge
G R Gibson
J H Cummings
G T Macfarlane
C Allison

Baragwanath Hospital, University of the Witwatersrand, Johannesburg, South Africa
I Segal

Department of Physiology, Potchefstroom University, Potchefstroom, South Africa
H H Vorster

South African Institute for Medical Research, Johannesburg, South Africa
A R P Walker

Correspondence to:
Dr G R Gibson, MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB2 1QL.

Accepted for publication
4 September 1989

The aerobic metabolism of carbohydrate in mammalian cells requires oxygen as the terminal electron acceptor and produces carbon dioxide, water, and energy as the principal end products. In anaerobic systems such as the lumen of the human colon, however, starch, non-starch polysaccharides (dietary fibre), and other substrates are fermented by the resident microflora to yield short chain fatty acids, carbon dioxide, hydrogen, and energy.[1]

Molecular hydrogen is used by intestinal methanogenic bacteria in many animal species to reduce carbon dioxide to methane. Methanogenic bacteria have been identified in man[2 3] but population studies have shown that as few as 21% (Indian adults) or as many as 95% (rural black African teenagers) excrete methane in breath.[4] Similarly, there is great variation in the production of hydrogen when subjects are challenged with standard doses of fermentable carbohydrate such as lactulose.[5]

Sulphate reducing bacteria are a group of obligately anaerobic bacteria which utilise sulphate as an oxidising agent for the dissimilation of organic matter – that is, replacing oxygen in conventional aerobic respiration. Molecular hydrogen can also act as an electron donor for dissimilatory sulphate reduction.[6] The major end product of this process is sulphide, which is rapidly hydrolysed to H_2S. In vitro studies have shown that in the large gut, sulphate reducing bacteria can outcompete methanogenic bacteria for limited amounts of hydrogen if sufficient sulphate is available.[7] A third role whereby molecular hydrogen can be metabolised in the colon is by the reduction of carbon dioxide to acetate, or homoacetogenesis.[8]

Thus, a number of pathways exist for the disposal of reducing equivalents formed during colonic fermentation. Competition for hydrogen among methanogenic bacteria, sulphate reducing bacteria, acetogenic bacteria, and other species probably occurs. This is important to man since removal of hydrogen by the large intestinal microflora shifts fermentation to more oxidized end products so increasing energy yield.[9] Little is known about these processes in the colon. We therefore measured rates of hydrogen accumulation, sulphate reduction, methanogenesis, and acetogenesis in faecal samples from 30 subjects to determine whether a competitive relation exists between these processes.

Methods

SUBJECTS

Fresh faecal samples were collected from 30 healthy volunteers (15 men and 15 women) aged 28–60 years. Twenty samples were taken from African subjects in the village of Hekpoort, Western Transvaal and 10 samples were from staff of the South African Institute of Medical Research in Johannesburg. Faeces were collected in the morning before being flown overnight to London for processing in Cambridge the following afternoon. No subject had taken antibiotics for at least eight weeks before giving a specimen. Samples were transported at ambient temperature as initial studies showed that freezing the faeces in 10% (w/v) glycerol significantly decreased hydrogen production and sulphate reducing activity (Table I).

TABLE I *Effect of different storage treatments upon hydrogen production and sulphate reducing activity in faeces. Values are mean (SEM)*

Treatment	Rate of hydrogen accumulation* (nmol/g dry wt/d)	Rate of sulphate reduction† (nmol/g dry wt/d)	Viable count SRB (log_{10}/g dry wt faeces)
Fresh faeces	72·5 (13·2)	321·8 (36·4)	9·0 (1·6)
Ambient temperature for 24 h	54·6 (11·6)	281·2 (29·4)	9·0 (1·2)
Freezing in 10% w/v glycerol	3·5 (0·9)	146·7 (28·4)	6·0 (1·7)

*9 hour incubation; †48 hour incubation. SRB=sulphate reducing bacteria.

BREATH METHANE

Duplicate end-expiratory breath samples were collected into 20 ml plastic syringes and methane was determined by gas chromatography using a Pye Unicam PU-4500 with flame ionisation detector. Samples of room air were also taken and the value subtracted from the breath sample. A methane producer was defined as a subject with more than 1 ppm methane in breath above values in ambient air.

The study was approved by the Ethical Committee of the Medical School of the University of the Witwatersrand, Johannesburg.

FAECAL SLURRIES

Faecal slurries (5% w/v) were prepared by homogenising samples in anaerobic sodium phosphate buffer (0·1 mol/l, pH 7·0). For measurements of hydrogen production and methanogenic, sulphate reduction, and acetogenic rates, Lintner's starch (0·2% w/v) was incorporated into the slurries as the fermentable substrate.

METHANE FORMATION RATES

Methane production from faecal slurries was measured as described by Allison and Macfarlane.[10] The linear part of the methane production plot during a 48 hour incubation period was used to calculate the rate of methanogenesis.

SULPHATE REDUCTION RATES

Triplicate sub-samples (5 ml) were removed from each faecal slurry and 5 μl volumes of carrier free sodium (^{35}S) sulphate added. Samples were then incubated anaerobically for 18 hours at 37°C before freezing and subsequent distillation using the method of Jørgensen.[11] Rates of sulphate reduction were calculated from the amount of acid volatile (^{35}S) sulphide formed.[12]

ACETATE FORMATION RATES

Rates of acetogenesis in 5 ml sub-samples from faecal slurries were determined in triplicate using the method of Jones and Simon.[13]

ENUMERATION OF SULPHATE REDUCING BACTERIA

Viable populations of sulphate reducing bacteria were counted using the agar shake dilution method with acetate, lactate, propionate, butyrate, and H_2/CO_2 as electron donors[6] since various studies[6 14 15] have shown that these are the major substrates that support the growth of sulphate reducing bacteria. Dilution tubes were incubated anaerobically at 37°C for 14 days. After this time, growth of sulphate reducing bacteria was indicated by a precipitation of ferrous sulphide and the number of black colonies formed was counted.

ISOLATION AND CHARACTERISATION OF SULPHATE REDUCING BACTERIA

Single colonies were removed from the highest agar shake dilution tubes and subcultured into liquid media. Pure cultures were obtained by successive passage through agar shakes and sulphate reducing bacteria were characterised using the criteria of Keith et al.[16]

HYDROGEN SULPHIDE CONCENTRATIONS

Values of H_2S in faeces were measured (after precipitation of sulphides in 10% w/v zinc acetate) using the spectrophotometric method of Cline.[17]

EFFECT OF pH UPON METHANOGENESIS, DISSIMILATORY SULPHATE REDUCTION, AND ACETOGENESIS IN FAECAL SLURRIES

To test whether colonic pH could significantly influence rates of methanogenesis, sulphate reduction, and acetogenesis, faecal slurries were prepared as described and adjusted to a range of pH values (5·5–8·5 in 0·5 increments). Methanogenic and acetogenic rates were calculated as before and sulphate reducing activity was determined by production of H_2S.

Results

TRANSPORT OF FAECAL SAMPLES

Two approaches were tested to assess the most favourable method of transporting faecal samples to the UK for processing. Faeces incubated at ambient temperature for 24 hours under an atmosphere of oxygen-free nitrogen as well as samples frozen in a slurry (5% w/v) containing 10% w/v glycerol were prepared. Rates of hydrogen production and sulphate reducing activity were subsequently determined and compared with those found in fresh faeces. Data presented in Table I show that activities were always reduced in the incubated and frozen samples. The percentage inhibition of hydrogen release, sulphate reduction, and numbers of viable sulphate reducing bacteria, however, were appreciably greater in the frozen samples. Faeces were therefore transported from South Africa at ambient temperature in sealed plastic bags.

TABLE II *Hydrogen metabolism in faecal samples from 30 healthy subjects. Results are mean (SEM)*

	No	Breath methane (ppm)	Rate of hydrogen accumulation* (nmol/g dry wt/d)	Rate of methane production† (nmol/g dry wt/d)	Rate of sulphate reduction† (nmol/g dry wt/d)	Rate of acetogenesis† (nmol/g dry wt/d)	Amount of H_2S in faeces (mM)
Group A	23	20·1 (3·7)	162 (25·1)	7902 (1061)	9·2 (4·3)	59·0 (8·4)	0·05 (0·01)
Group B	7	0	154 (32·1)	0	1478 (661)	108 (14·3)	0·21 (0·03)

Group A=number of sulphate reducing bacteria <10⁷/g dry wt faeces.
Group B=number of sulphate reducing bacteria >10⁷/g dry wt faeces.
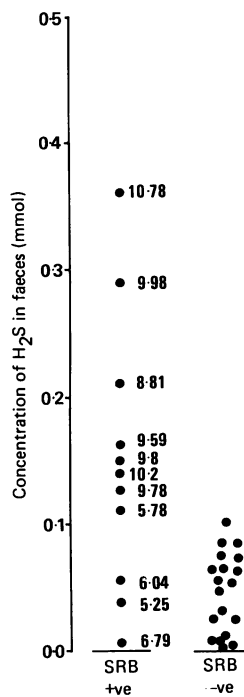*9 hour incubation; †48 hour incubation.

Figure 1: Viable counts of sulphate reducing bacteria (SRB) in relation to hydrogen sulphide values in human faeces. Numbers indicate $\log_{10} SRB/g$ dry weight faeces.

TABLE III Percentage distribution of sulphate reducing bacteria (SRB) in human faecal samples. Results are mean (SEM)

Carbon source	Percentage SRB utilising each substrate
Acetate	14·1 (7·0)
Lactate	63·1 (24·1)
Propionate	9·4 (2·3)
Butyrate	9·2 (2·8)
H_2/CO_2	4·3 (1·6)

HYDROGEN ACCUMULATION, METHANOGENESIS, SULPHATE REDUCTION/AND ACETOGENESIS IN FAECAL SLURRIES

When faecal slurries were incubated with Lintner's starch a small amount of hydrogen accumulated during the first nine hours of incubation. The rate of hydrogen release during this time did not differ greatly in any of the samples tested (Table II). After nine hours, hydrogen concentrations gradually declined. To determine the route of hydrogen uptake during this period, rates of methanogenesis, sulphate reduction, and acetogenesis were measured and compared in each of the faecal slurries.

On the basis of methanogenesis rates and numbers of sulphate reducing bacteria (SRB) in faeces, the subjects divided readily into two groups (Table II). Most subjects (group A; n=23) shared high rates of faecal methanogenesis and had less than 10^7 SRB/g dry weight faeces. In group A, 21 of the 23 subjects had methane in the breath. None of the subjects in group B (n=7) had methane in the breath, produced methane in vitro, or had more than 10^7 SRB/g of faeces. Group B subjects had high rates of sulphate reduction in faeces and higher concentrations of sulphide. Low rates of sulphate reduction and H_2S formation were detected in some samples from group A but these were much less than those measured in the group B subjects (Table II).

Viable populations of sulphate reducing bacteria were enumerated with acetate, lactate, propionate, butyrate, and H_2/CO_2 as electron donors to give total counts of faecal sulphate reducing bacteria. Sulphate reducer counts showed a strongly positive association with H_2S concentrations in faeces (Fig 1). Hydrogen sulphide values in the four group A faecal samples that contained less than 10^7 SRB/g were similar to those in which sulphate reducing bacteria were completely absent.

Rates of acetogenesis were relatively low in all samples tested (Table II). Those in group B, however, were approximately double those found in group A suggesting that acetate produc-

tion from H_2/CO_2 may be more important in non-methanogenic subjects.

ISOLATION AND CHARACTERISATION OF SULPHATE REDUCING BACTERIA

The highest numbers of sulphate reducing bacteria were found using lactate as a source of carbon and energy (Table III). The dominant bacteria consisted of curved rods of various sizes, identified as belonging to the genus *Desulfovibrio*.[18] *Desulfovibrio* species do not have a complete TCA cycle and acetate is therefore a normal end product of their metabolism. As a consequence, coccobacillary rods, identified as the acetate oxidizing *Desulfobacter* species[19][20] were occasionally isolated from lactate-containing tubes. In dilution tubes containing acetate as the sole carbon source, *Desulfobacter* species were always numerically predominant. When propionate was the electron donor, the major bacteria isolated belonged to the genus *Desulfobulbus*[21] which incompletely oxidizes propionate to acetate and carbon dioxide. Large non-spore forming rod shaped bacteria identified as *Desulfotomaculum acetoxidans*[22][23] were isolated from butyrate enrichments with *Desulfovibrio* species and *Desulfobulbus* species generally present with H_2/CO_2 as a substrate.

INFLUENCE OF pH UPON METHANOGENIC, SULPHATE REDUCING, AND ACETOGENIC ACTIVITY

Data presented in Table IV show that the optimal pH values for methanogenesis and dissimilatory sulphate reduction were 7·0 and 7·5 respectively. At acidic pH values both processes were substantially inhibited whereas homoacetogenesis was highest at relatively low pH values.

Discussion

Dietary carbohydrate that escapes digestion and absorption in the small bowel passes into the caecum where fermentation by anaerobic bacteria occurs producing hydrogen.[24] A number of fates for this hydrogen then exist. A small proportion may pass through the gut wall into the blood and be transported to the lungs where it is then excreted in breath.[25][26] Alternatively, hydrogen can be metabolised by the large intestinal microflora. The removal of hydrogen allows a depletion of electron sink products such as lactate, succinate, and ethanol resulting in a higher energy yield from fermentation.[9] Thus adequate removal of H_2 allows a more complete recovery of energy by bacteria from the degradation of organic substrates. An appreciable proportion of hydrogen may be removed by the action of methanogenic,[27] sulphate reducing,[28] and homoacetogenic bacteria.[29][30] All three processes remove four moles of hydrogen per mole CO_2 or SO_4^{2-} reduced.

Hydrogen is essential for the growth of colonic methanogens[2][3][31] and if this substrate is removed methanogenesis cannot occur. In the present study, considerable methane production occurred only when sulphate reducing bacteria were not active – that is group A subjects (Table II).

TABLE IV Effect of pH upon methanogenesis, sulphate reduction, and acetogenesis in faecal slurries. Results are mean (SEM) of triplicate determinations

pH	Rate of methanogenesis (nmol/g dry wt/h)	Rate of H_2S production (nmol/g dry wt/h)	Rate of acetogenesis (nmol/g dry wt/h)
5·5	10·2 (3·2)	3·8 (0·7)	1·84 (0·04)
6·0	31·2 (8·1)	11·9 (6·2)	1·93 (0·06)
6·5	43·6 (7·1)	13·4 (5·3)	4·65 (0·8)
7·0	57·2 (8·9)	25·7 (5·3)	0·83 (0·1)
7·5	54·2 (9·4)	28·9 (4·1)	0·69 (0·1)
8·0	44·3 (5·2)	27·4 (4·9)	0·28 (0·09)
8·5	21·4 (3·1)	10·4 (2·7)	0

The metabolic end product of dissimilatory sulphate reduction (H_2S) is thought to be toxic to methanogenic bacteria,[32-34] but at the low concentrations measured in faeces (Fig 1), it will not exert any direct inhibitory effect. When sulphate is available, sulphate reducing bacteria are known to have a higher substrate affinity for hydrogen than methanogenic bacteria,[35-37] and this is a more likely explanation for the apparent mutual exclusion of methanogenesis and sulphate reduction in the large gut. We have shown that sulphate reducing bacteria outcompete methanogenic bacteria for hydrogen when faecal slurries from methane and non-methane producing subjects are mixed together.[7]

In the group A samples, low rates of sulphate reduction and H_2S production occurred even in the presence of active methanogenesis. It is likely that the small amount of sulphate reduced is by assimilation into sulphur-containing amino acids and subsequently proteins. The H_2S produced in faeces from group A subjects was probably released from these amino acids during protein fermentation. Four of the group A subjects had low numbers of sulphate reducing bacteria in faeces but H_2S values were similar to those found in group B samples, so these sulphate reducers were active at values that did not affect hydrogen uptake by methanogenic bacteria. Some sulphate reducing bacteria can grow fermentatively in the absence of sulphate[28] and in this case methanogenic bacteria may act as important hydrogen scavengers to keep concentrations below thermodynamically unfavourable values.[38 39] Sulphate reducing bacteria would then function as hydrogen producing acetogens.[29]

An alternative route for hydrogen disposal is by reduction of CO_2 to acetate. A recent study has indicated that this may occur in man.[8] Homoacetogenesis involves the utilisation of hydrogen and carbon dioxide to form acetate via acetyl CoA.[40] Homoacetogenic bacteria are, however, competitively displaced by methanogenic bacteria for available hydrogen in other anaerobic ecosystems.[41 42] Thus, these bacteria will only become active when there is little hydrogen uptake by sulphate reducing or methanogenic bacteria, explaining the low rates of acetogenesis recorded in this study. The fact that rates were generally higher in the non-methanogenic (group B) slurries may be linked to the concentration of available sulphate. If sulphate is limited and hydrogen is in relative excess, some of the hydrogen remaining after sulphate reduction could then be available for metabolism by homoacetogenesis (some other unknown factor would have to limit methanogenesis, however).

The concentration of sulphate present in the large gut is therefore critical for determining which of these processes occurs. If sufficient sulphate exists, sulphate reducing bacteria will predominate. If the colonic sulphate pool is low, however, these bacteria will not utilise appreciable amounts of hydrogen. During these conditions, methanogenic bacteria or perhaps acetogenic bacteria will become important. Recent studies have shown that a large variability in the amount of sulphate reaching the colon exists.[43] The contribution of endogenous sulphate is approximately 1 mmol/d whereas dietary levels
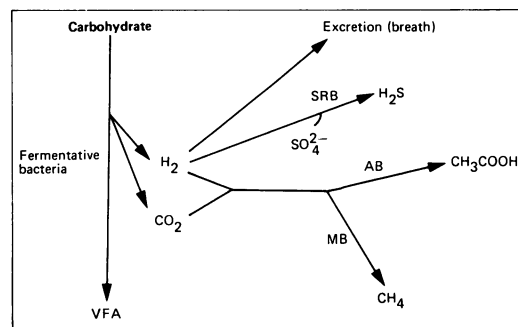

Figure 2: Postulated mechanisms for hydrogen disposal in the human colon.
SRB=sulphate reducing bacteria; MB=methanogenic bacteria; AB=acetogenic bacteria; VFA=volatile fatty acids.
Carbohydrate which reaches the colon is fermented by populations of anaerobic bacteria producing VFA and the gases H_2 and CO_2. A small proportion of the H_2 is excreted.
The remainder can then undergo further metabolism. If sufficient sulphate exists, SRB are primarily responsible and H_2S is produced. During conditions of low sulphate availability, however, MB and AB are able to combine H_2 with CO_2 to form methane and acetate respectively.

can range from 2–16 mmol/d, with maximal absorption occurring below 7 mmol/d.

Colonic pH may also be an important factor controlling the rate of hydrogen uptake in the large intestine. The right colon, where most carbohydrate fermentation occurs, is a region of low pH whereas conditions in the left colon and sigmoid rectum areas frequently approach neutrality.[44] Homoacetogenesis may become important at low pH in the colon, because in vitro studies showed that faecal sulphate reducing and methanogenic bacteria were relatively pH-sensitive, preferring an environment that is neutral or slightly alkaline, whereas highest rates of acetogenesis occurred at acidic pH values (Table IV). Furthermore, we have shown previously using a three chambered fermentation system that at a pH of 6·0, hydrogen uptake can occur without any appreciable contribution from sulphate reducing or methanogenic bacteria.[45]

A number of possible pathways for disposal of H_2 exist therefore in man and are summarised in Figure 2. What are the clinical consequences of this? Firstly, it makes it highly unlikely that simple relations can be drawn between fermentation of specified substrates, such as lactulose, and H_2 evolution in breath. In practice, widely differing responses to standard oral doses of fermentable carbohydrate are seen among subjects.[5] Bjorneklett and Jenssen[46] have shown that subjects who produce methane during fermentation produce appreciably less H_2 in breath in response to a standard dose of lactulose. Secondly, if H_2 is not further metabolised, fermentation may be incomplete and intermediates such as lactate, succinate, and ethanol are likely to accumulate. D-lactate, produced by colonic bacteria, is only partly metabolised in man and can cause severe metabolic disturbance on occasions.[47] Thirdly, if H_2 gas is not metabolised the volume of gas accumulating in the gut will be substantially greater than if CH_4 is produced because the reaction:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$
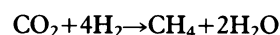
reduces five volumes of gas to one. Fourthly, the end products of these various terminal oxidative reactions differ in their toxicity. Methane is a harmless gas, readily expelled. Acetate is absorbed and metabolised by peripheral tissue such as muscle but H_2S is highly toxic and may poison colonic epithelial cells if not oxidised rapidly after absorption. The capacity for high rates of H_2S production exists in some people (Table II) and it may be that sulphate reducing bacteria play a part in the aetiology of some large gut disorders. We have previously shown,[7] using in vitro faecal slurries, that up to 3 mM H_2S may be produced during a 48 hour incubation. In this study faecal H_2S did not rise above a concentration of 0·4 mM (Fig 1). This suggests that some detoxification mechanism for H_2S is operative in the large gut. Such a mechanism may include incorporation into sulphide containing amino acids or the production of mercaptans – for example, mercaptoacetate or mercaptobutyrate.

1 Cummings JH. Fermentation in the human large intestine: evidence and implications for health. *Lancet* 1985; i: 1206–8.

2 Miller TL, Wolin MJ, Macario EC de, Macario AJL. Isolation of *Methanobrevibacter smithii* from human feces. *Appl Env Microbiol* 1982; 43: 227–32.

3 Miller TL, Wolin MJ. Stability of *Methanobrevibacter smithii* populations in the microbial flora excreted from the human large bowel. *Appl Env Microbiol* 1983; 45: 317–8.

4 Segal I, Walker ARP, Lord S, Cummings JH. Breath methane and large bowel cancer risk in contrasting African populations. *Gut* 1988; 29: 608–13.

5 Flourie B, Florent C, Etanchaud F, *et al*. Starch absorption by healthy men evaluated by lactulose hydrogen breath test. *Am J Clin Nutr* 1988; 47: 61–6.

6 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.

7 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: 241–7.

8 Lajoie SF, Bank S, Miller TL, Wolin MJ. Acetate production from hydrogen and (^{14}C) carbon dioxide by the microflora of human feces. *Appl Env Microbiol* 1988; 54: 2723–7.

9 Wolin MJ. Interactions between H_2-producing and methane-producing species. In: Schlegel HG, Gottschalk G, Pfennig N, eds. *Microbial formation and utilization of gases*. Gottingen: Goltze Press, 1976: 141–50.

10 Allison C, Macfarlane GT. Effect of nitrate upon methane production by slurries of human faecal bacteria. *J Gen Microbiol* 1988; 134: 1397–405.

11 Jørgensen BB. A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments. I. Measurement with radiotracer techniques. *Geomicrobiol J* 1978; 1: 11–27.

12 Sorokin YI. Experimental investigation of bacterial sulfate reduction in the Black Sea using S^{35}. *Microbiologica* 1962; 31: 329–35.

13 Jones JG, Simon BM. Interactions of acetogens and methanogenesis in anaerobic freshwater sediments. *Appl Environ Microbiol* 1985; 49: 944–8.

14 Laanbroek HJ, Pfennig N. Oxidation of short chain fatty acids by sulfate-reducing bacteria in freshwater and in marine sediments. *Arch Microbiol* 1981; 128: 330–5.

15 Sørensen J, Christensen D, Jørgensen BB. Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediment. *Appl Environ Microbiol* 1981; 42: 5–11.

16 Keith SM, Herbert RA, Harfoot CG. Isolation of new types of sulphate-reducing bacteria from estuarine and marine sediments using chemostat enrichments. *J Appl Bacteriol* 1982; 53: 29–33.

17 Cline JD. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol Oceanogr* 1969; 14: 454–8.

18 Postgate JR, Campbell LL. Classification of *Desulfovibrio* species, the non-sporing sulphate-reducing bacteria. *Bacteriol Rev* 1966; 30: 732–8.

19 Widdel F, Pfennig N. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. I. Isolation of new sulfate-reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov., sp. nov. *Arch Microbiol* 1981; 129: 395–400.

20 Widdel F. New types of acetate-oxidizing, sulfate-reducing *Desulfobacter* species, *D. hydrogenophilus* sp. nov., *D. latus* sp. nov., and *D. curvatus* sp. nov. *Arch Microbiol* 1987; 148: 286–91.

21 Widdel F, Pfennig N. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. Incomplete oxidation of propionate by *Desulfobulbus propionicus* gen. nov., sp. nov. *Arch Microbiol* 1982; 131: 360–5.

22 Widdel F, Pfennig N. A new anaerobic sporing acetate-oxidizing sulfate-reducing bacterium, *Desulfotomaculum acetoxidans* (emend). *Arch Microbiol* 1977; 112: 119–22.

23 Widdel F, Pfennig N. Sporulation and further nutritional characteristics of *Desulfotomaculum acetoxidans*. *Arch Microbiol* 1981; 129: 401–2.

24 Calloway DH, Colasito DJ, Matthews RD. Gases produced by human intestinal microflora. *Nature* 1966; 212: 1238–9.

25 Levitt MD. Production and excretion of hydrogen gas in man. *N Engl J Med* 1969; 281: 122–7.

26 Levitt MD, Ingelfinger FJ. Hydrogen and methane production in man. *Ann NY Acad Sci* 1968; 150: 75–81.

27 Balch WE, Fox GE, Magrum LJ, Woese CR, Wolfe RS. Methanogens: reevaluation of a unique biological group. *Microbiol Rev* 1979; 43: 260–96.

28 Postgate JR. *The sulphate-reducing bacteria*. 2nd edn. Cambridge: Cambridge University Press, 1984.

29 Fuchs G. CO_2 fixation in acetogenic bacteria: variations on a theme. *FEMS Microbiol Rev* 1986; 39: 181–213.

30 Thauer RK, Jungermann K, Decker K. Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol Rev* 1977; 41: 100–80.

31 Miller TL, Wolin MJ. *Methanosphaera stadtmaniae* gen. nov., sp. nov.: a species that forms methane by reducing methanol with hydrogen. *Arch Microbiol* 1985; 141: 116–22.

32 Cappenberg TE. Interrelations between sulphate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. I. Field observations. *Ant van Leeuwen J Microbiol Serol* 1974; 40: 285–95.

33 Cappenberg TE. Interrelations between sulphate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. II. Inhibition experiments. *Ant van Leeuwen J Microbiol Serol* 1974; 40: 297–306.

34 Cappenberg TE. A study of mixed continuous cultures of sulphate-reducing and methane-producing bacteria. *Microbiol Ecol* 1975; 2: 60–72.

35 Kristjansson JK, Schönheit P, Thauer RK. Different K_s values for hydrogen of methanogenic bacteria and sulfate-reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate. *Arch Microbiol* 1982; 131: 278–82.

36 Lovley DR, Dwyer DF, Klug MJ. Kinetic analysis of competition between sulfate reducers and methanogens for hydrogen in sediments. *Appl Environ Microbiol* 1982; 43: 1373–9.

37 Lupton FS, Zeikus JG. Physiological basis for sulfate dependent H_2 competition between sulfidogens and methanogens. *Curr Microbiol* 1985; 11: 7–12.

38 McInerney MJ, Bryant MP. Anaerobic degradation of lactate by syntrophic associations of *Methanosarcina barkeri* and *Desulfovibrio* species and effect of H_2 on acetate degradation. *Appl Environ Microbiol* 1981; 41: 346–54.

39 Bryant MP, Campbell LL, Reddy CA, Crabile MR. Growth of *Desulfovibrio* on lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. *Appl Environ Microbiol* 1977; 33: 1162–9.

40 Coutts DAP, Balba MTM, Senior E. Acetogenesis and acetotrophy in a hexanoate-catabolizing microbial association isolated from anoxic landfill. *J Appl Bacteriol* 1987; 63: 343–53.

41 Prins RA, Lankhors A. Synthesis of acetate from CO_2 in caecum of some rodents. *FEMS Microbiol Lett* 1977; 1: 255–8.

42 Zeikus JG. Microbial populations in digestors. In: Stafford DA, Wheatley BI, Hughes DE, eds. *Proceedings of 1st international symposium on anaerobic digestion*. London: Applied Science, 1980: 75–103.

43 Florin THJ, Neale G, Cummings JH. Dietary and endogenous sulphate losses from the upper gastrointestinal tract. *Proc Med Res Soc Summer Meeting* 1989: 22.

44 Cummings JH, Pomare EW, Branch WJ, Naylor CPE, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987; 28: 1221–7.

45 Gibson GR, Cummings JH, Macfarlane GT. Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Appl Environ Microbiol* 1988; 54: 2750–5.

46 Bjorneklett A, Jenssen F. Relationship between hydrogen (H_2) and methane (CH_4) production in man. *Scand J Gastroenterol* 1982; 17: 985–92.

47 Oh MS, Phelps KR, Traube M, Barbosa-Salvidor JL, Boxhill C, Cavioli HJ. D-Lactic acidosis in a man with short-bowel syndrome. *N Engl J Med* 1979; 301: 249–52.