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INFLUENCE OF DIET ON MICROBIAL PRODUCTION AND UTILIZATION OF H_2, CH_4, H_2S IN THE COLON: PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL CONSEQUENCES

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SUMMARY

The intestinal microflora is capable of degrading and fermenting complex oligosaccharides and amino acids generating short chain fatty acids (mostly acetate, propionate, butyrate). The colonic microflora also produces copious amounts of H_2 and CO_2 during fermentation of carbohydrates. Varying amounts of H_2S and CH_4 are produced by the intestinal microflora and in humans appear to be strongly correlated with dietary habits. A diet high in red meat favours the formation of increased amounts of H_2S. In contrast, a diet low in meat and high in resistant starch favours the formation of CH_4 and increased amounts of short chain fatty acids. A high meat diet also shifts the bile acid pool in man to primarily taurine conjugation. Taurine is another source of colonic H_2S. Sulphate reducing bacteria have been shown to out-compete methane producing bacteria for available H_2 both *in vitro* and *in vivo*. In humans, *Desulfovibiro vulgaris* and *Methanobrevibacter smithii* are believed to be the bacterial species most responsible for the formation of H_2S and CH_4, respectively. H_2S, in high concentrations (mM) in the colon, appears to be highly toxic and carcinogenic to colonocytes and may contribute to the pathogenesis of inflammatory diseases and colon cancer. In high concentrations, H_2S inhibits the mitochondrial terminal respiratory chain and decreases the metabolism of butyrate by colonocytes. However, intestinal mucosal cells have the ability to detoxify H_2S by oxidative metabolism in the mitochondria and conversion to thiosulphate by rhodanese. H_2S can be synthesized endogenously from cysteine in mammalian cells and may function as a gasotransmitter molecule controlling the opening of K_{ATP} channels in smooth muscle cells, neurons, cardiomyocytes and pancreatic β-cells. The formation of H_2S by intestinal bacteria and metabolism by host cells provides an excellent example of how the body flora and diet can impinge on host physiology and pathophysiology.

INTRODUCTION

The first life on earth evolved in an anoxic environment. For 2.5 billion years, microorganisms have successfully exploited niches devoid of molecular oxygen (*Brocks* et al., 1999). In fact, it is interesting to note that one of the most densely inhabited environments on earth is the mammalian large

intestine (>10^{11} bacteria/gram wet weight faeces) (*Moore* et al., 1974; *Whitmann* et al., 1998). To put this in perspective, the adult human body contains an order of magnitude more prokaryotic cells (10^{14}) than mammalian cells (10^{13}). The collective bacterial genomes (termed "microbiome") of the human microflora encode an estimated 2-4 million genes, surpassing the human genome by a staggering 100-fold (*Bäckhed* et al., 2005). Intestinal bacteria can carry out hundreds, if not thousands, of enzymatic reactions not carried out by host cells. Hence, the human body should be regarded as a complex ecosystem of interacting prokaryotic and eukaryotic cells balanced by selective pressures from both the "top-down" (host) and the "bottom-up" (gut flora) (*Ley* et al., 2006). *Relman* and *Falkow* (2001) have called for the task of sequencing this microbiome, which they have termed "the second human genome project". We will, however, focus only on a small fraction of the genes within this "microbiome", and illustrate the magnitude of effect these few metabolic pathways have on human well-being and how diet appears to control them.

A few species of eubacteria and archaea have evolved the ability to utilize hydrogen gas and carbon dioxide waste products of colonic bacterial fermentation. Metabolism of molecular hydrogen by the microbial community improves the thermodynamics of colonic fermentation, which in turn provides the host with more energy and comfort through reduced gas volume. Three pathways exist in colonic bacteria to oxidize hydrogen, including: acetogenesis, methanogenesis, and sulphidogenesis. An important observation gathered from review of the current literature on the physiology of hydrogen sulphide is that concentration determines whether this gas functions as a toxic compound or "gasotransmitter". Hydrogen sulphide has come to be a recognized "gasotransmitter" along with CO and NO produced by the host for normal physiological processes. This was in fact quite a surprise given the toxic nature of sulphide. However, the gastrointestinal flora produces large amounts of hydrogen sulphide in some individuals. Given appropriate dietary and endogenous sulphur sources, the gastrointestinal flora can tip the balance toward disease states through copious production of H_2S in the human large bowel. In this review, we will examine the importance of diet on routes of hydrogen metabolism in the human colon in relation to colon cancer incidence and examine the native African case study as a model for diet, colonic bacterial metabolism and colorectal cancer risk.

THE GASTROINTESTINAL ECOSYSTEM

The human colon is a hollow organ roughly 1.5 m in length, 6.5 cm in diameter with a volume of 540 ml (*Wilson*, 2005). The colonic epithelium secretes mucin, a sulphated glycoprotein, which polymerizes into a viscous gel increasing in thickness from ±107 μM (proximal colon) to ±155 μM (rectum) along the length of the large bowel. The mucosal surface functions largely as an innate defence against microbial colonization of the epithelium, binding toxic metabolites, and provides lubrication that aids in peristaltic movement of luminal contents (*Wilson*, 2005). The colon functions to absorb water and recover energy from undigested dietary material through bacterial me-

tabolism. From the ileum, a mixture of undigested dietary material, biliary and pancreatic secretions known collectively as chyme empty into the caecum. As chyme enters the caecum, a sharp decrease in pH relative to the ileum (from pH 7.5 in the ileum to pH 5.7 in the caecum) occurs due to rapid bacterial fermentation of undigested carbohydrates and production of short chain fatty acids (SCFA; see next section). pH rises along the length of the colon to roughly 6.8 in the rectum as a result of absorption of SCFA by the colonic epithelium and buffering by bicarbonate secretion. Unlike the lumen of the small intestine, which supports mainly facultative anaerobes (10^6 to 10^8 bacteria.ml^{-1}), the lumen of the colon is strictly anaerobic with redox potentials ranging from -200 mV to -300 mV. For this reason bacterial biotransformations in the colon are largely restricted to hydrolytic and reductive reactions.

The host is sterile before birth, though bacteria are acquired during and shortly after birth (*Savage*, 1977) initially from maternal contact (*Mandar* and *Mikelsaar*, 1996; *Conway*, 1995) followed by continuous environmental exposure which persists throughout life. The composition of the gut flora appears to be assembled through factors such as environment, host genetics and stochastic events or "historical contingencies" such as colonization order (*Dethlefsen* et al., 2006). The adult flora is largely established during weaning, once the diet shifts to solid food (*Conway*, 1995). Factors such as redox potential, bile salt concentration, pH, transit time, available dietary substrates and host genotype relating to binding sites and secreted host factors produce strong selective pressures on potential bacterial colonizers. This is evident in light of recent comprehensive 16S rDNA sequencing and comparison of the human gut flora between individuals (*Eckburg* et al., 2005, *Ley* et al., 2006). Of the 50 bacterial phyla represented, only two major lineages, the Firmicutes and the Bacteroidetes are significantly represented in the gut flora of humans (>98% of the sequences) (*Eckburg* et al., 2005, *Ley*, 2006). In addition, only a single Archaean, *Methanobrevibacter smithii*, was represented in 1524 archaeal 16S rDNA sequences (*Eckburg* et al., 2005). This archaean has been shown to be the predominant methanogen in human faeces in other molecular based (*Lin* and *Miller*, 1998) and culture based studies (*Miller* and *Wolin*, 1982, 1983, *Miller* et al., 1984). The human colonic Firmicutes and Bacteroidetes lineages terminate in broad, shallow radiations comprising an estimated 800 species and likely thousands of strains (*Backhed* et al., 2005, *Dethlefsen*, 2006, *Ley*, 2006).

The majority of gut inhabitants are metabolically versatile generalists as a result of both microbe-microbe competition and synthrophy (*Dolfing* and *Gottschal*, 1996) as well as "top-down" selection favoring stable communities with functional redundancies that provide the host a steady supply of nutrients despite variations in diet (*Ley* et al., 2006). The 'normal' flora protect against infection through saturating binding sites that otherwise could be exploited by pathogens (*Ley* et al., 2006). Metabolic cross-feeding sets up competitive as well as cooperative interactions between intestinal microbes (*Flint* et al., 2007). For instance, species of *Roseburia* produce hydrogen and butyrate during acetate consumption. In co-culture with an acetogen, hydrogen is consumed, producing acetate which is then metabolized to butyrate, which is absorbed by the host or further metabolized by colonic bacteria (*Chassard* and *Bernalier-Donadille*, 2006).

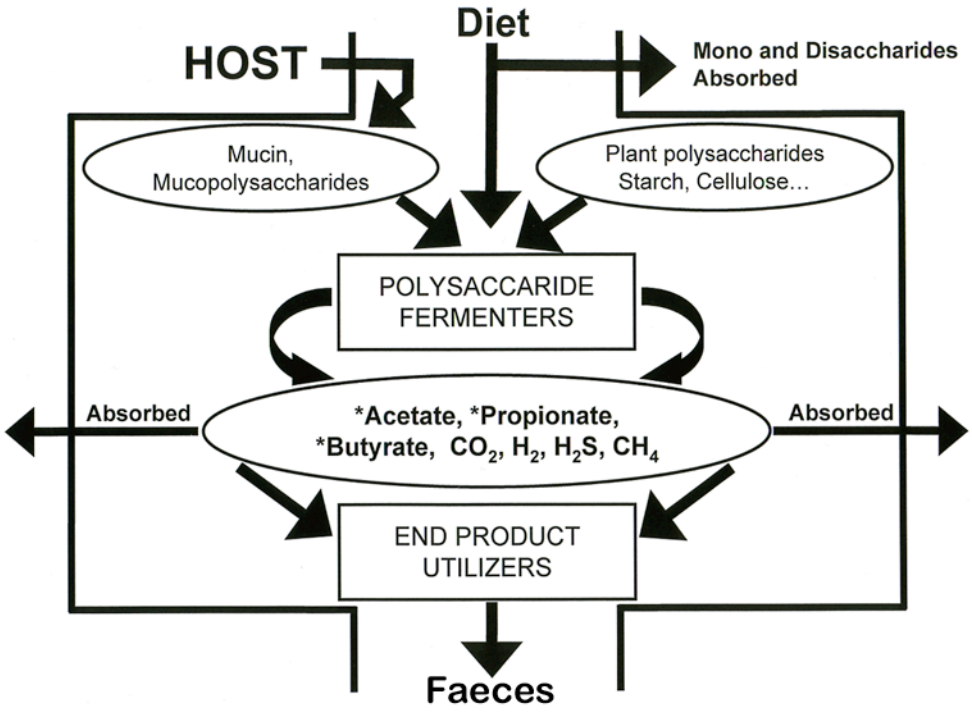

Figure 1: Growth substrates for colonic bacteria. Sloughed intestinal epithelial cells (100-200 g/day wet weight), plant polysaccharides and resistant starches are major substrates for colonic bacteria. Colonic bacteria produce short chain fatty acids (acetate, propionate, and butyrate 5:3:1 ratio) and various gases (CO_2, H_2, CH_4) from these substrates. Short chain fatty acids are absorbed from the colon and are metabolized by various tissues in the body. The amounts of different gases produced are influenced by dietary habits.

METABOLISM BY THE 'SECOND HUMAN GENOME'

Carbohydrates are the major and preferred carbon and energy source for the gut microflora. Indeed, the genus most represented in the human large intestine, the *Bacteroides* (*Wilson*, 2005), appears to dominate due to their arsenal of glycosylases (*Sonnenburg* et al., 2006). In fact, *Bacteroides thetaiotaomicron* alone contains 128 more glycoside hydrolases than the human host genome (*Xu* et al., 2003). Fermentation of complex dietary and endogenously produced carbohydrates is an emergent property of the microbiota (Figure 1). Breakdown of complex polysaccharides produces oligosaccharides and monosaccharides substrates which can be fermented by other members of the flora resulting in a chemical food web (*Hudson* and *Marsh*, 1995). Fermentation end products include the major short chain fatty acids (SCFA) acetate, propionate and butyrate in roughly a 5:2:1 ratio as well as the gases CO_2 and H_2 (Figure 1) (*Hoverstad* et al., 1984; *Miller* and *Wolin*, 1979). SCFA account for 5-8% of the total caloric intake/day in humans, which may seem irrelevant given the problem of "overeating" in many industrialized nations; however, the colonic epithelium derives an estimated 70% of its energy

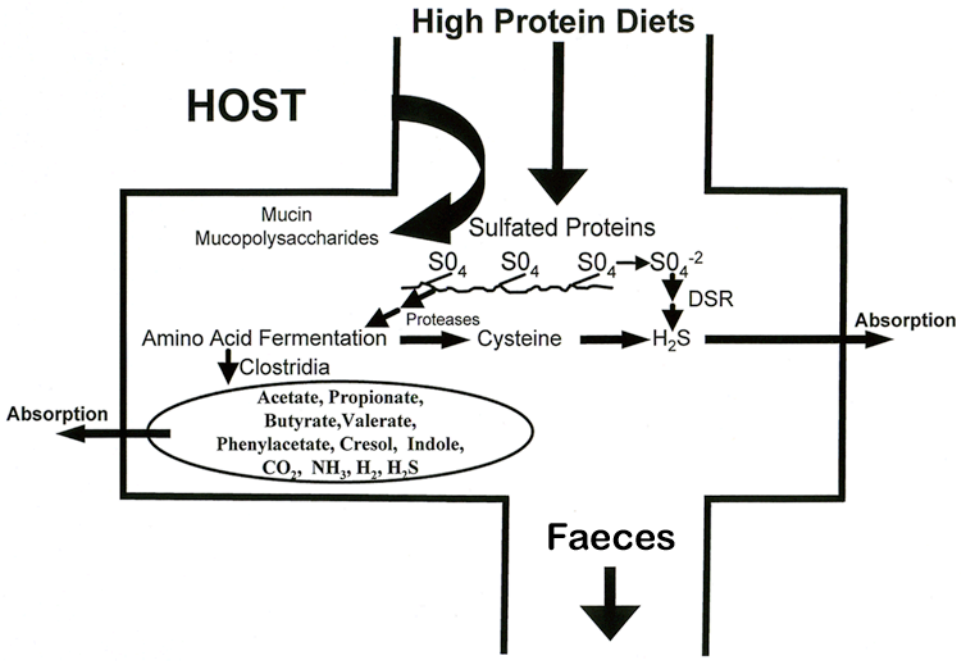

Figure 2: Effect of high protein diets on colonic bacterial metabolism. Increased input of protein into the colon increases the amount of hydrogen sulphide produce by colonic bacteria and selects for dissimilatory sulphate reducing (DSR) bacteria. High protein diets may also select for amino acid fermenting bacteria i.e. *Clostridium species*. The types and amounts of short chain fatty acids produced are altered by high protein diets.

from butyrate oxidation (*Bergman*, 1990). Indeed, this is an interesting example of co-evolution in which colonocyte gene regulation is geared toward β-oxidation of a microbial fermentation product. In addition, the amount of each fatty acid produced can be altered by many factors, including dietary habits. For example, a diet high in resistant starch produces more total SCFAs and more butyrate (*Scheppach* et al., 1988).

Metabolism of dietary protein generally occurs in the distal colon as carbohydrates become limiting. Proteolysis also represents a significant source of SCFA, H_2 and CO_2 production in the colon (*Macfarlane* and *Macfarlane*, 1995). Unlike saccharolytic fermentation, many additional and potentially harmful metabolites are produced from the fermentation of amino acids by clostridia via the Stickland reaction. This pathway of energy metabolism involves the oxidation/reduction of pairs of amino acids. During this process, ATP is generated via substrate level phosphorylation (*Lengeler* et al., 1999). Reaction products resulting from amino acid fermentation include 3-methylbutyrate, 4-methylvalerate, phenylacetate, cresol, indole acetate, indole, formamide, amines, and hydrogen sulphide (*Macfarlane* and *Macfarlane*, 1995) (Figure 2). In this regard, diets high in animal fat and protein have been linked to several cancers including breast, prostate and colon cancer (*Bingham*, 1999).

Additional substrates for the gut flora include the approximately 100-200 grams of sloughed intestinal

epithelial cells that enter the human colon each day along with bile components (Figure 1). Indeed, breakdown of host mucin supports a diverse colonic flora through production of oligosaccharides, monosaccharides, SCFA and free SO_4^{-2} (*Macfarlane* et al., 1989; *Gibson* et al., 1988; *Corfield* et al., 1992). Some members of the flora specialize in use of alternative electron acceptors i.e. carbon dioxide (methanogens and acetogens), free sulphate from diet and mucin degradation (sulphate reducing bacteria), cholesterol, bile acids (7α-dehyroxylation), steroid hormones, bilirubin and others. Indeed, specialized functions have been targeted to achieve a clinical outcome. The most familiar example is the use of prebiotics to increase the levels and activity of *Bifidobacteria, which* specifically metabolize fructo-oligosaccharides (*Gibson* et al., 1995). Thus, bacteria have evolved to utilize a wide range of host dietary and endogenous substrates with considerable functional redundancy, cross-feeding as well as specialization.

HYDROGENOTROPHIC METABOLISM

Hydrogen gas represents a key metabolite whose partial pressure regulates the SCFA profile through thermodynamic control of substrate oxidation (*Hungate*, 1967; *Wolin* and *Miller*, 1983). Hydrogen gas is formed as a means of disposing of reducing equivalents during fermentation, and at elevated partial pressures inhibits oxidation of reduced NADH, a coenzyme essential for hexose catabolism (*Gibson* et al., 1990b). Therefore, removal of molecular hydrogen is important both to the flora and the host as higher energy yields are derived through more efficient fermentation (*Gibson* et al., 1993). H_2, unlike SCFA's, is not metabolized by host cells, and instead the host expels 50-60% through breath and flatus (*Christl* et al., 1992). It has been observed that far less hydrogen is excreted than would be expected theoretically during fermentation of carbohydrates, suggesting that interspecies hydrogen transfer is the predominant mechanism of hydrogen gas disposal (*Christl* et al., 1992).

In aquatic ecosystems, hydrogen is consumed through two primary pathways: Methanogenesis and dissimilatory sulphate-reduction (DSR). Numerous studies in anoxic natural environments have shown that given adequate sulphate, methanogens are effectively out-competed for hydrogen (*Widdel*, 1988; *Cappenburg*, 1974a,b; *Martens* and *Berner*, 1974; *Winfrey* and *Zeikus*, 1977; *Oremland* and *Taylor*, 1978). Hydrogenotrophic dissimilatory sulphate-reduction proceeds through the following equation: $SO_4^{-2} + H^+ + 4H_2 \rightarrow HS^- + H_2O$ ($\Delta G^{\circ\prime} = -152$ kJ/mol HS^-). Hydrogenotrophic methanogenesis proceeds as follows: $CO_2 + 4 H_2 \rightarrow CH_4 + 2H_2O$ ($\Delta G^{\circ\prime} = -131$ kJ/mol CH_4) (*Lengeler* et al., 1999). Sulphate reducing bacteria can outcompete methanogens as DSR by hydrogen gas yields more cellular energy than methanogensis. In the absence of sulphate, methanogenesis predominates (*Cappenberg* et al., 1974a,b) as under normal conditions, copious amounts of CO_2 and molecular hydrogen are present.

Similar to other natural anoxic environments, reduction of H_2 gas volume in the colon is carried out by a small group of hydrogenotrophic methanogenic archaea (MA) which are predominantly represented by *Methanobrevibacter smithii* (*Miller* and *Wolin*,

1983; *Miller* et al., 1984; *Lin* and *Miller*, 1998; *Eckburg* et al., 2005) as well as hydrogenotrophic sulphate-reducing bacteria (SRB) including *Desulfovibrio spp.* and *Desulfotomata spp.* (*Postgate* and *Campbell*, 1966; *Holdeman* et al., 1976; *Gibson* et al., 1988b,c; *Gibson* et al., 1990a; *Willis* et al., 1997). *In vitro* and *in vivo* studies have convincingly demonstrated a competitive relationship between human faecal populations of SRB and MA (*Gibson* et al., 1988a,b,c; *Gibson* et al., 1990a; *Christl* et al., 1992). Indeed, both groups of organisms have been shown to predominate in the distal gut, thus their overlapping spatial distribution should provide competitive microenvironments for molecular hydrogen. *Gibson* et al. (1988b) constructed a three-stage continuous culture system (TSCCS) to mimic the dilution rate, pH and substrates found in various regions of the colon to test the hypothesis that methanogenesis could be inhibited by sulphate sources. Previous observations in sudden death victims suggested MA preferentially inhabit the distal colon (*Macfarlane* et al., 1992). Indeed, methanogenesis was detected predominantly in vessel 3 of the TSCCS (distal colon) as expected due to considerations of pH. However, upon addition of 5.8 g/day porcine gastric mucin, methanogenesis was strongly inhibited with a concomitant 100-fold increase in DSR rates and an increase in SRB levels by several $\log_{10}$ colony-forming units. Once mucin addition was terminated sulphide production and SRB counts decreased and methanogenesis recovered to baseline. *Christl* et al. (1992) studied the *in vivo* effect of sulphate consumption on methanogenesis and sulphidogenesis in healthy humans placed on a low-sulphate diet. Hydrogen metabolism in 3 of 6 methanogenic individuals changed in response to addition of sodium sulphate to the diet. Breath methane decreased significantly along with methanogen colony counts, while SRB increased from undetected to 10^3 g^{-1} wet weight and sulphide production rate increased 3-fold. These observations changed the perception that methanogenesis was stable and independent of diet (*Bond* et al., 1971). It appears that methanogenesis can be rapidly and steadily inhibited through competition for molecular hydrogen with SRB and that dietary and endogenous sulphate sources regulate these relationships *in vivo*. The amount of sodium sulphate given in the *Christl* et al. (1992) study was 1.6 mmol; however, western dietary intake is estimated to vary between 1.5 and 16.0 mmol (*Florin* et al., 1993). Therefore, significant amounts of sulphide are routinely consumed in the western diet which may result in sulphidogenic hydrogen metabolism.

It has been observed by several studies that SRB and MA are not mutually exclusive in methanogenic individuals, so the question arises how SRB maintain growth rates to prevent washout. Desulfovibrios are found both in the lumen as well as mucosal associated, and may be able to subsist at low growth rates in the mucus (*Fite* et al., 2004). A second possibility suggests that rather than competing, methanogens may actually facilitate the persistence of SRB in the absence of adequate sulphate. A syntrophic relationship exists between *Methanobrevibacter smitthii* and *Desulfovibrio* spp. in the human colon in the absence of sulphate (*Stolyar* et al., 2007). In the absence of sulphate sources, *Desulfovibrio spp.* will ferment substrates such as lactate, which under high partial pressures of hydrogen, are extremely energetically unfavourable (*McInerney* and *Bryant*, 1981). In this regard, methanogenesis reduces hydrogen par-

tial pressure and makes Desulfovibrio fermentation of organic acids thermodynamically favourable. Indeed, levels of sulphate-reducing bacteria in some "methanogenic" individuals is low (~10^2 SRB.gram^{-1} wet weight) vs. 10^6 to 10^8 SRB.gram^{-1} wet weight in sulphidogenic populations (*Gibson* et al., 1988c). In this sense, the sulphate-reducer, in order to maintain status as a "resident" (autochthonous) member of the flora, must compete for organic acids with other secondary metabolizers (*Willis* et al., 1997). Methanogenesis may thus permit carriage of SRB. However, input of a selective agent (sulphate in this case) appears to break the synthrophy between the methanogen and SRB and allows H_2 dependent growth of *Desulfovibrios* with significant and rapid increases in abundance-especially if significant quantities of sulphate persists (*Gibson* et al., 1988b; *Christl* et al., 1992). While this has been shown both *in vitro* and *in vivo*, recent data have shown that some individuals harbour high levels of both SRB and MA (*Pitcher* et al., 2000; *Levine* et al., 1998; *Lewis* and *Cochrane*, 2006). Therefore, the relationship may be more complicated in certain situations and may depend on the composition of the gut flora, hydrogen partial pressures and possibly unidentified factors.

Acetogenesis is a third form of hydrogen disposal in the gut of mammals which forms acetate from H_2 and CO_2 through acetyl-CoA (*Lengeler*, 1999). Acetogenesis follows this equation: $4H_2 + 2\ CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$ ($\Delta G^{\circ\prime} = -94.9$) (*Lengeler* et al. 1999). This pathway requires the same molar ratio of H_2 as methanogenesis and sulphidogenesis but yields the least amount of energy and will therefore be out-competed by both methanogenesis and sulphate reduction at the near neutral pH values in the distal colon. There have been some reports of acetogenesis in human faeces (*Lajoie* et al., 1988; *Bernalier* et al., 1996; *Leclerc* et al., 1997). Addition of $^{13}CO_2$ to faecal suspensions of three human subjects resulted in $^{13}CH_3COO^-$ only in the low methanogen (<10^2 g^{-1} dry weight) and non-methanogenic faeces. However, methanogenesis and sulphidogenesis appear mainly in the distal colon where pH is optimal. At acidic pH, in the caecum and ascending colon, acetogenesis may occur (*Gibson* et al., 1995). However, these same organisms are also saccharolytic, and would likely ferment sugars over acetogenesis for energetic reasons. While playing a role in hydrogenatrophic metabolism in some individuals, the majority of humans produce either methane or sulphide. The route of interspecies hydrogen transfer may have significant consequences for human health.

SULPHIDE AND DISEASE: A HYPOTHESIS

Several lines of evidence implicate sulphide production and SRB in the pathogenesis of the inflammatory bowel disease ulcerative colitis (UC). Faecal sulphide has been shown to be significantly elevated in UC patients with active disease (*Christl* et al., 1995; *Pitcher* et al., 1995; *Gibson* et al., 1991; *Roediger* et al., 1997) while other studies reported no significant increase (*Moore* et al., 1998). Stool measurements likely do not reflect total production of sulphide but rather binding capacity of chelators in stool. Roughly 95% H_2S is estimated to be absorbed through the colon (*Levitt* et al., 2002). Thus, faecal measurements underestimate the amount of sulphide

exposed to and detoxified by the colonic mucosa. Indeed, due to multiple dissociation states (HS^- and H_2S) and pH values in the colon, once H_2S is formed about 2/3 dissociates into the HS^- anion. However, H_2S, and to a lesser degree HS^-, are permeable to the plasma membrane of colonocytes. Because the relatively high concentration of H_2S in the colon (mM) as compared to blood (50 μM) there is a natural diffusion gradient into colonocytes.

Sulphide has been shown in several studies to inhibit butyrate oxidation, the preferred energy pathway for the colonic epithelium both *in vitro* (*Roediger* et al., 1980; *Roediger* and *Nance*, 1986; *Babidge* et al., 1998) and *in vivo* (*Roediger*, 1980). Indeed, breath CO_2 and luminal bicarbonate were significantly reduced in UC patients as compared to controls after rectal instillation of butyrate (*Roedinger* et al., 1984; *Den Hond* et al., 1996). In addition, sulphide increased proliferation in upper colonic crypts by 54% in one study, and this proliferation was reversed by butyrate (*Christl* et al., 1996). *In vivo* evidence demonstrates the effectiveness of butyrate enemas on the clinical symptoms of UC (*Harig* et al., 1989; *Sheppach* et al., 1992). This data strongly suggests that UC is an "energy deficiency" disease whose pathology is rooted in impaired β-oxidation (*Roedinger*, 1980). *Roedinger* et al. (1997) pointed out that impairment of butyrate metabolism would inhibit processes critical to maintaining epithelial cell barrier function. A noted risk factor for relapse into active UC is high meat intake, which provides substrate for SRB and thus sulphide production (*Tragnone* et al., 1995; *Magee* et al., 2000). In addition, SRB numbers and activity are significantly upregulated during active vs. quiescent disease (*Pitcher* et al., 2000). Treatment of UC with 5-aminosalicyclic acid (5-ASA) containing drugs including sulphasalazine resulted in inhibition of sulphide production in the colon and improvement of symptoms (*West* et al., 1974; *Pitcher* et al., 2000). In addition, animals fed sulphated polysaccharides (carageenan or dextran sodium sulphate) developed colitis, which could be inhibited by antibiotic treatment (*Onderdonk* et al., 1978) and fails to induce colitis in germfree animals (*Onderdonk* et al., 1977) suggesting the importance of the colonic flora in UC. However, while this is a compelling hypothesis, it has yet to be determined whether the increase in H_2S in patients with inflammatory bowel disease precedes the disease or is an alteration of the normal microflora as a result of chronic inflammation.

Inflammatory bowel disease significantly increases risk of developing colon cancer (*Mayer* et al., 1999; *Dincer* et al., 2007; *Tanaka* et al., 2006). Colonocytes have protective enzymes (rhodanese and thiomethyltransferase) which are expressed at the mucosal surface functioning to detoxify H_2S produced by colonic bacteria. Rhodanese (thiosulphate sulphurtransferase) can catalyze the formation of thiosulphate from H_2S and SO_3^{-2} and appears to be the main enzyme involved in detoxification (Figure 3). Studies from *Levitt* and co-workers (1999) showed that the addition of $H_2{}^{35}S$ to colonic mucosa resulted in the formation of primarily $^{35}S^{35}SO_3^{-2}$. The effect of H_2S on colonocyte physiology and pathophysiology is a function of concentration. At low concentrations (μM), H_2S can be oxidized by the mitochondria electron transport chain via H_2S:quinone oxidoreductase, an enzyme originating in eubacteria exposed to sulphidic environments (*Theissen* et al., 2003; *Goubern* et al., 2007). The input of electrons increases the electrochemical gradient of the cytoplasmic

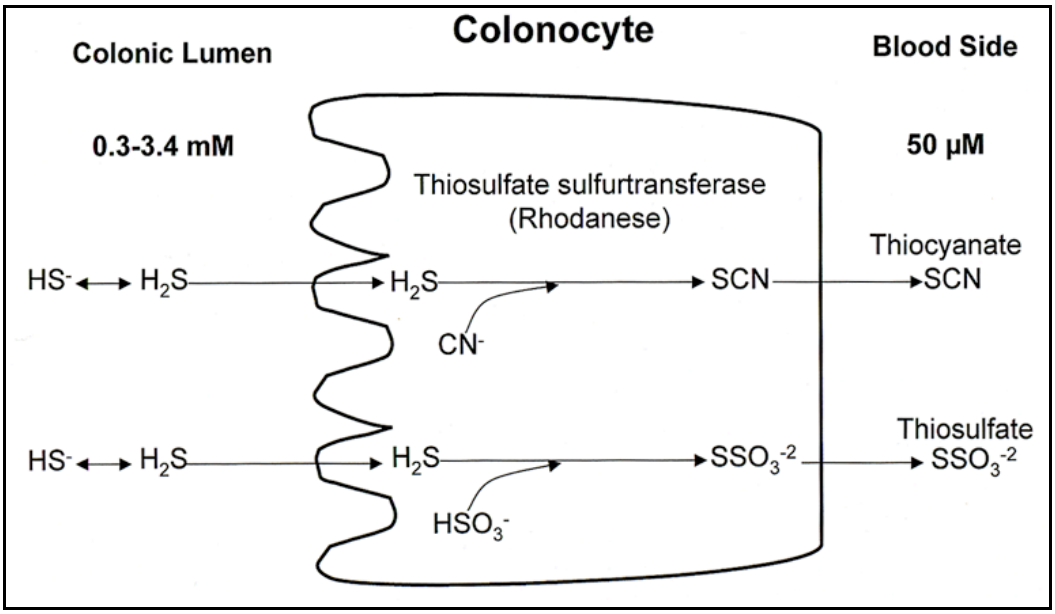

Figure 3: Effect of high meat vs. high resistant starch diets on colonic bacterial metabolism. High meat diets increase the amount of total protein and taurine conjugated bile acids (BA) entering the colon which serve as substrates for hydrogen sulphide formation by gut bacteria. Diets high in resistant starches (RS) and low in meat produce more methane and less hydrogen sulphide (H_2S) than high meat diets. In addition, diets high in RS produces more short chain fatty acids (SFA) as compared to high meat diets.

membrane of mitochondria and can increase ATP synthesis. The oxidation of H_2S generates various oxidation products of sulphur including: $S_2O_3^{-2}$, HSO_3^-, SO_4^{-2}. Enzymatic metabolism of H_2S and SO_3^{-2} by rhodanese generates thiosulphate, which is eliminated in urine (*Morris* and *Murer*, 2001). In contrast, at high concentrations (mM) of H_2S there is marked inhibition of cytochrome oxidase and a decrease of the mitochondria electrochemical gradient inhibiting basic cellular physiology including production of oxidized sulphur for sulphide detoxification by rhodanese.

Interestingly, recent data suggests loss of sulphide-detoxification possibly occurs in active UC and CRC (*Ramasamy* et al., 2006) and functional genetic polymorphisms of rhodanese (*Billaut-Laden* et al., 2006) coupled with dietary habits in some individuals (*Magee* et al., 2000) may saturate and overwhelm rhodanase enzymatic activity, leading to persistent conditions conducive to genotoxicity which lead to genetic changes and ultimately CRC. Indeed, recent data suggests that hydrogen sulphide is involved in the carcinogenesis process either through direct DNA damage or through upregulation of signalling pathways leading to proliferation, loss of apoptosis and tumour vascularization (*Attene-Ramos* et al., 2006, 2007; *Deplancke* et al., 2003; *Rose* et al., 2005).

HYDROGEN SULPHIDE AS A GASOTRANSMITTER

Hydrogen sulphide appears to be the third gasotransmitter synthesized in mammalian cells along with nitric oxide (NO) and carbon monoxide (CO). NO and CO are formed by NO synthase and haeme oxygenase, respectively. Hydrogen sulphide can function as a neuromodulator, smooth muscle relaxant and can play a role in protecting the intestinal mucosa (*Distrutti* et al., 2006; *Fiorucci* et al., 2006). Mammalian cells synthesize H_2S primarily by the activity of two highly regulated enzymes: cystathionine-γ-lyase (CSE) and cystathionine-β-synthetase (CBS). These two pyridoxal phosphate-dependent enzymes are differentially expressed in tissues throughout the body. For example, the brain contains large amounts of CBS, whereas CSE is highest in peripheral tissues. The levels of H_2S in tissue and blood are highly regulated and are in the micromolar range (30-160 μM). Unlike the high concentrations (mM) of H_2S formed in the colon by intestinal bacteria, the levels of H_2S form in tissues are probably not toxic under normal physiological conditions. However, there is a small concentration range where H_2S functions as a regulatory gas that is not toxic.

Hydrogen sulphide appears to evoke many different physiological responses in mammalian cells (for reviews see *Wang* 2002, 2003; *Lowicka* and *Beltowski*, 2007). The cellular and molecular mechanisms regulated by H_2S are beginning to be elucidated. Hydrogen sulphide has been reported to regulate various cell signalling cascades and specific ion channels. The effect of H_2S on ATP-sensitive potassium channels (K_{ATP}) has been well studied. Many cellular effects of H_2S can be mimicked by drugs (pinacidil) that are agonist for K_{ATP} channels and these effects can be blocked by K_{ATP} channel antagonists (glibenclamide). H_2S appears to play a protective role against gastric injury caused by anti-inflammatory non-steroidal drugs (NSAIDs) (*Fiorucci* et al., 2005). NSAIDs were found to reduce cystathione-γ-lyase (CSE) mRNA and protein resulting in a decrease in H_2S formation in gastric mucosa. The addition of exogenous NaHS restored protection of the mucosa by increasing blood flow, inhibiting leukocyte adherence to endothelial cells and repressing pro-inflammatory cytokine formation induced by NASIDs. The protective effects of NaHS were through its effects on K_{ATP} channels.

Hydrogen sulphide has been reported to regulate various cell signalling pathways in mammalian cells including: ERK (stimulation and inhibition), iNOS (stimulation and inhibition), increase in intracellular Ca^{2+}, stimulation of adenylate cyclase among others (*Lowicka* and *Beltowski*, 2007, *Zhi* et al., 2007). There is no evidence that H_2S activates guanylyl cyclase as does NO. Moreover, H_2S can chemically react with reactive oxygen and nitrogen species, which may also alter different, cell signalling pathways. There is evidence that there may be cross-talk between H_2S and NO generation in mammalian cells (*Lowicka* and *Beltowski*, 2007).

COLORECTAL CANCER: THE NATIVE AFRICAN CASE STUDY

In the United States, colorectal cancer (CRC) is expected to be the third leading cause of cancer death in both men and women in 2007 in the United

States (*American Cancer Society*, 2007). However, patients classified as high-risk for colon cancer including those with familial adenomatous polyposis (FAP), hereditary non-polyposis CRC or inflammatory bowel disease comprise only 5-15% of all CRC incidence (*O'Shaughnessy* et al., 2002). The majority of CRC incidence is thus nonhereditary and sporadic; suggesting the importance of environmental influences. Epidemiologically, CRC incidence is primarily found in "Western" nations who consume a diet high in animal fat and protein (*Parkin* et al., 1992). In particular, native black Africans have been observed to have extraordinarily low colon cancer rates (*Burkitt*, 1971) as compared to African Americans who have higher incidence than Caucasian Africans or Caucasian Americans (*American Cancer Society*, 2005; *O'Keefe* et al., 1985, 2007). *Berg* (1973) made the observation that CRC risk increased significantly in descendents of immigrant populations from low-risk nations (including native Africans) living in developed nations who adopted a "Western" diet. It was originally thought that the low incidence of CRC observed in native black Africans was due to dietary fibre (*Burkitt*, 1971). However, adoption of a more 'Westernized' diet has not led to an increase in CRC despite lower non-starch fibre intake than the majority of 'Western' nations (*O'Keefe* et al., 1985; *Segal*, 2002). Within the same region, whites, despite better overall nutrition, including high non-starch fibre intakes have approximately a 10-fold higher rate of CRC (*Sitas* and *Parcella*, 1989). Recent data has called into question the hypothesis that non-starch fibre intake is associated with low CRC risk (*Park* et al., 2005; *Fuchs* et al., 1999). In fact, two large prospective studies demonstrated that dietary fibre supplementation failed to significantly reduce adenomatous polyp recurrence (*Schatzkin* et al., 2000; *Alberts* et al., 2000). Therefore, a closer look at the behaviour of the African population is required. Two important observations have been made in native African populations:

1) Native Africans consume large quantities of maize, which is high in resistant starch rather than non-starch fibre; and three times less red meat than South African whites (*O'Keefe* et al., 1999), and

2) This population absorbs carbohydrates in the small bowel with decreased efficiency as compared to other ethnic groups resulting in increased resistant starch reaching the large bowel (*Segal* et al., 2002; *O'Keefe* et al., 1999).

In addition, and as noted above, this population has the highest rates of methanogenesis (90%) as compared to Caucasian British (30%) and low sulphidogenesis in native Africans (15% vs. 70% in British) (*Gibson* et al., 1988c), and some of the lowest rates of diverticular disease and inflammatory bowel disease. The consumption of foods high in resistant starch and low in animal protein and fat was concluded to be a contributing factor to low incidence of colon cancer in native Africans relative to South African whites and indeed the remainder of the Western world (*O'Keefe* et al., 1999). The question remains as to a potential mechanism, an explanation of this data that may hold the simple key to reducing the risk of colonic disease. A recent animal model of high meat vs. resistant starch diet in mice coupled with genome-wide transcriptome analysis of *Bacteroides thetaiotaomicron* in a gnotobiotic animal model may help to explain the Native African data in terms of diet and our microbiome.

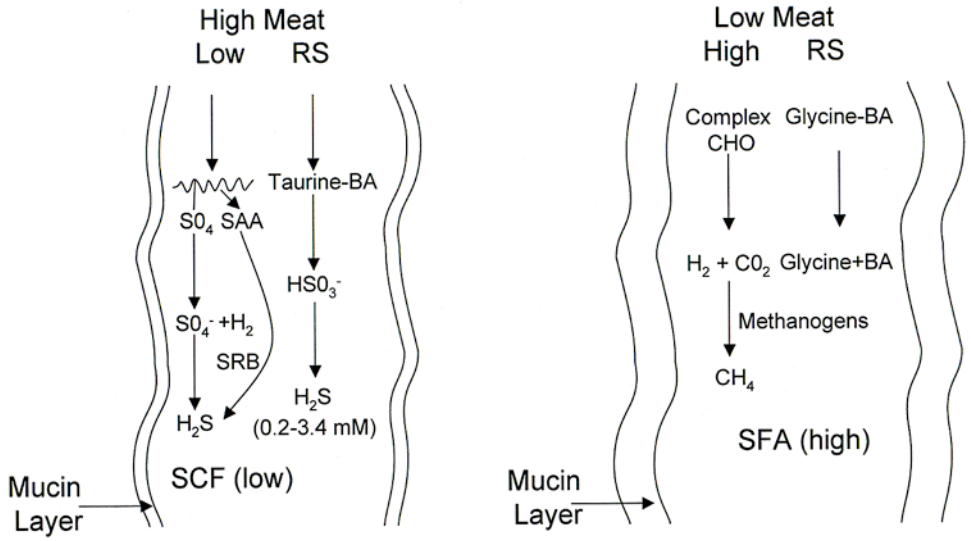

Figure 4: Metabolism of hydrogen sulphide by coloncytes. Hydrogen sulphide (H_2S) produced by gut bacteria can be taken up by colonocytes and metabolized to thiosulphate and thiocyanate by rhodanese. High levels of rhodanese protein and activity are found in colonic tissue.

CONSILIENCE

The title of this section is derived from the classic work of Edward O. Wilson entitled *Consilience,* which he expressed as "a unity of knowledge". A disease as complex as colon cancer is clearly multifactorial and requires a unity of knowledge from several fields. In this review the data has led us to examine aspects of the biology in all three domains of life; the eubacteria, the archaebacteria and the eukarya. We will now provide a synthesis of this analysis in light of exciting recent studies.

The observations gathered from studies in South Africa led Toden and co-workers to develop an animal model in order to determine the link between red meat, resistant starch and colorectal cancer (*Toden* et al., 2006, 2007). These studies demonstrated that resistant starch prevented DNA damage and mucin depletion in a dose-dependent manner. In the absence of RS, mucin was depleted 40% in rat colons on a meat diet and colonocyte DNA damage was nearly twice as great in the absence of RS as compared to its presence. Mucin may be important for binding toxic molecules, whose depletion may lead to cellular damage. DNA damage correlated negatively with butyrate production (*Toden* et al., 2007). Butyrate is the preferred source of energy for the colonocyte and regulates normal cellular functions (*Bergman*, 1990). Two possible explanations exist for the observed decrease in colonic mucus in meat fed rats. The first possibility is that SCFA increase mucin secretion through intestinal epithelial MUC2 gene expression (*Willemsen* et al., 2003), however, other studies have reported conflicting results (*Tarrerias* et al., 2002). A second interpretation is induction of mucin degrading genes by intestinal bacteria. A gnotobiotic mouse model of glycan foraging by *Bacteroides thetaiotaomicron* addressed genome-wide transcriptional

changes and metabolic products of this bacterium during abrupt changes in the carbohydrate composition of the mouse diet (complex polysaccharides vs. simple sugars). In the presence of complex dietary carbohydrates, *B. thetaiotaomicron* expressed genes involved in the attachment, uptake and metabolism of these compounds. However, when the diet shifted to simple carbohydrates that are absorbed by the host, and thus do not reach the colon, the microbe shifted its transcriptome to genes involved in degrading host mucin (*Sonnenburg* et al., 2005). These data suggest that in the absence of sufficient dietary carbohydrates, host mucus provides a stable source of carbohydrates (*Ridlon* and *Hylemon*, 2006) (Figure 4). Mucus thickness exists in equilibrium between host production and losses due to mechanical abrasion and bacterial metabolism.

As noted previously, utilization of host mucin results in release of large amounts of free sulphate, which the *Bacteroides spp*. cannot utilize. Thus along the intestinal tract, sulphate levels begin to increase until reaching the left colon where *Desulfovibrio spp.* reside. Their levels can rapidly rise in response to this substrate resulting in production of cytotoxic and potentially carcinogenic hydrogen sulphide. Lifelong exposure to high colonic sulphide levels due to diets low in complex polysaccharides and high in meat may decrease mucin thickness, increase distal colon putrifaction and secondary bile acid levels resulting in constant exposure of the colonic epithelium to molecules which up-regulate cell-turnover and intracellular production of DNA damaging reactive oxygen species. Conditions may be set up to generate mutations in colonocytes that may lead to colon cancer; possibly more rapidly if the sulphide-detoxifying pathway is impaired as is observed in UC and CRC (*Ramasamy* et al., 2006). The native African population appears to maintain a stable colonic environment through consumption of complex carbohydrates in the diet resulting in a steady supply of butyrate, precluding mucin degradation by supplying colonic bacteria with dietary carbohydrates, down-regulation of bacterial sulphate-reduction and lowering production of carcinogenic nitrogen compounds through their method of cooking and low animal protein and fat consumption.

LITERATURE

American Cancer Society: Cancer facts and figures. American Cancer Society, Atlanta (2007).

Attene-Ramos, M.S., Wagner, E.D., Gaskins, H.R., and Plewa, M.J.: Hydrogen sulfide induces direct radical-associated DNA damage. Mol. Cancer Res. 5, 455-459 (2007).

Attene-Ramos, M.S., Wagner, E.D., Plewa, M.J., and Gaskins, H.R.: Evidence that hydrogen sulfide is a genotoxic agent. Mol. Cancer Res. 4, 9-14 (2006).

Babidge, W., Millard, S., and Roediger, W.: Sulfides impair short chain fatty acid beta-oxidation at acyl-CoA dehydrogenase level in colonocytes: Implications for ulcerative colitis. Mol. Cell. Biochem. 181, 117-124 (1998).

Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., and Gordon, J.I.: Host-bacterial mutualism in the human intestine. Science 307, 1915-1920 (2005).

Berg, A.: Nutrition, development and population growth. Popul. Bull. 29, 3-37 (1973).

Bergman, E.N.: Energy contributions of volatile fatty acids from the gastrointestinal

tract in various species. Physiol. Rev. 70, 567-590 (1990).

Bernalier, A., Lelait, M., Rochet, V., Grivet, J.P., Gibson, G.R., and Durand, M.: Acetogenesis from H_2 and CO_2 by methane- and non-methane producing human colonic bacterial communities. FEMS Microbiol. Ecol. 19, 193-202 (1996).

Billaut-Laden, I., Allorge, D., Crunelle-Thibaut, A., Rat, E., Cauffiez, C., Chevalier, D., Houdret, N., Lo-Guidice, J., and Broly, F.: Evidence for a functional genetic polymorphism of the human thiosulfate sulfurtransferase (Rhodanese), a cyanide and H_2S detoxification enzyme. Toxicology 225, 1-11 (2006).

Bingham, S.A.: High-meat diets and cancer risk. Proc. Nutr. Soc. 58, 243-248 (1999).

Brocks, J.J., Logan, G.A., Buick, R., and Summons, R.E.: Archean molecular fossils and the early rise of eukaryotes. Science 285, 1033-1036 (1999).

Burkitt, D.P.: Epidemiology of cancer of the colon and rectum. Cancer 28, 3-13 (1971).

Cappenberg, T.E.: Interrelations between sulfate-reducing bacteria and methane-producing bacteria in bottom deposits of a fresh-water lake. II. Inhibition experiments. Antonie Van Leeuwenhoek 40, 297-306 (1974a).

Cappenberg, T.E. and Prins, R.A.: Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. III. Experiments with ^{14}C-labelled substrates. Antonie Van Leeuwenhoek 40, 457-469 (1974b).

Chassard, C. and Bernalier-Donadille, A.: H_2 and acetate transfers during xylan fermentation between a butyrate-producing xylanolytic species and hydrogenotrophic microorganisms from the human gut. FEMS Microbiol Lett. 254, 116-122 (2006).

Christl, S.U., Murgatroyd, P.R., Gibson, G.R., and Cummings, J.H.: Production, metabolism and excretion of hydrogen in the large intestine. Gastroenterology 102, 1269-1277 (1992).

Christl, S.U., Eisner, H.D., Dusel, G., Kasper, H., and Scheppach, W.: Antagonistic effects of sulfide and butyrate on proliferation of colonic mucosa: A potential role for these agents in the pathogenesis of ulcerative colitis. Dig. Dis. Sci. 41, 2477-2481 (1996).

Christl, S.U., Scheppach, W., and Kasper, H.: Hydrogen metabolism in the large intestine-physiology and clinical implications. Z. Gastroenterol. 33, 408-413 (1995).

Conway, P.L.: Microbial ecology of the human large intestine. In: Human colonic bacteria: Role in nutrition, physiology, and pathology (Gibson, G.R., Macfarlane, G.T., Eds.). CRC Press, Boca Raton, 1-18 (1995).

Corfield, A.P., Wagner, S.A., Clamp, J.R., Kriaris, M.S., and Hoskins, L.C.: Mucin degradation in the human colon: Production of sialidase, sialate *O*-acetylesterase, *N*-acetylneuraminate lyase, arylesterase, and glycosulfatase activities by strains of fecal bacteria. Inf. Immun. 60, 3971-3978 (1992).

Den Hond, E., Hiele, M., Ghoos, Y., Rutgeerts, P.: *In vivo* colonic butyrate metabolism in extensive ulcerative colits. Gastroenterology 110, A797 (1996).

Deplancke, B. and Gaskins, H.R.: Hydrogen sulfide induces serum-independent cell cycle entry in nontransformed rat intestinal epithelial cells. FASEB J. 17, 1310-1312 (2003).

Dethlefsen, L., Eckburg, P.B., Bik, E.M., and Relman, D.A.: Assembly of the human intestinal microbiota. Trends Ecol. Evol. 21, 517-523 (2006).

Dincer, Y., Erzin, Y., Himmetoglu, S., Gunes, K.N., Bal, K., and Akcay, T.: Oxidative DNA damage and antioxidant activity in patients with inflammatory bowel disease. Dig. Dis. Sci. 52, 1636-1641 (2007)

Distrutti, E., Sediari, L., Mencarelli, A., Renga, B., Orlandi, S., Antonelli, E., Roviezzo, F., Morelli, A., Cirino, G., Wallace, J.L., and Fiorucci, S.: Evidence that hydrogen sulfide exerts antinociceptive effects in the gastrointestinal tract by activating K_{ATP} channels. JPET 316, 325-335 (2006).

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M.,

Gill, S.R., Nelson, K.E., and Relman, D.A.: Diversity of the human intestinal microbial flora. Science 308, 1635-1638 (2005).

Fiorucci, S., Distrutti, E., Cirino, G., and Wallace, J.L.: The emerging role of hydrogen sulfide in the gastrointestinal tract and liver. Gastroenterology 131, 259-271 (2006).

Fiorucci S., Antonelli E., Distrutti E., Rizzo G., Mencarelli A., Orlandi S., Zanardo R., Renga B., Sante M.D., Morelli A., Cirino G., and Wallace J.L.: Inhibition of hydrogen sulfide generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs. Gastroenterology 129, 1210-1224 (2005).

Fite, A., Macfarlane, G.T., Cummings, J.H., Hopkins, M.J., Kong, S.C., Furrie, E., and Macfarlane, S.: Identification and quantification of mucosal and faecal desulfovibrios using real time polymerase chain reaction. Gut 53, 523-529 (2004).

Flint, H.J., Duncan, S.H., Scott, K.P., and Louis, P.: Interactions and competition within the microbial community of the human colon: Links between diet and health. Environ. Microbiol. 9, 1101-1111 (2007).

Florin, T.H.J., Neale, G., Gibson, G.R., Christl, S.U., and Cummings, J.H.: Metabolism of dietary sulphate: absorption and excretion in humans. Gut 32, 766-773 (1991).

Florin, T.H.J., Neale, G., Goreski, S., and Cummings, J.H.: The sulfate content of foods and beverages. J. Food. Anal. 6, 140-151 (1993).

Florin, T.H.J., Zhu, G., Kirk, K.M., and Martin, N.G.: Shared and unique environmental factors determine the ecology of methanogens in humans and rats. Am. J. Gastroenterol. 95, 2872-2879 (2000).

Fuchs, C.S., Giovannucci, E.L., Colditz, G.A., Hunter, D.J., Stampfer, M.J., Rosner, B., Speizer, F.E., and Willett, W.C.: Dietary fiber and the risk of colorectal cancer and adenoma in women. N. Engl. J. Med. 340, 169-176 (1999).

Gaskins, H.R., Gibson, G.R., Beatty, E.R., Wang, X., and Cummings, J.H.: Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 108, 975-982 (1995).

Gibson, G.R., Cummings, J.H., and Macfarlane, G.T.: Competition for hydrogen between sulphate-reducing bacteria and methanogenic bacteria from the human large intestine. J. Appl. Bacteriol. 65, 241-247 (1988a).

Gibson, G.R., Cummings, J.H., and Macfarlane, G.T.: 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. 54, 2750-2755 (1988b).

Gibson, G.R., Macfarlane, G.T., and Cummings, J.H.: Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. J. Appl. Bacteriol. 65, 103-111 (1988c).

Gibson, G.R., Cummings, J.H., Macfarlane, G.T., Allison, C., Sefal, I., Vorster, H.H., and Walker A.R.P.: Alternative pathways for hydrogen disposal during fermentation in the human colon. Gut 31, 679-683 (1990a).

Gibson, G.R., Cummings, J.H., and Macfarlane, G.T.: Factors affecting uptake by bacteria growing in the human large intestine. In: Microbiology and biochemistry of strict anaerobes involved in interspecies hydrogen transfer (Belaich, J.P., Bruschi, M., and Garcia, J.L., Eds.). Plenum Press, New York, 191 (1990b).

Gibson, G.R., Cummings, J.H., and Macfarlane, G.T.: Growth and activities of sulphate reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. FEMS Microbiol. Ecol. 86, 101-112 (1991).

Gibson, G.R., Macfarlane, G.T., and Cummings, J.H.: Sulphate-reducing bacteria and hydrogen metabolism in the human large intestine. Gut 34, 437-439 (1993).

Gouben, M., Andriamihaja, M., Nübel, T., Blachier, F., and Bouillaud, F.: Sulfide, the first inorganic substrate for human cells. FASEB J. 21, 1699-1706 (2007).

Harig, J.M., Soergel, K.H., Komorowski, R.A., and Wood, C.M.: Treatment of diversion colitis with short chain fatty acid irrigation. N. Engl. J. Med. 320, 23-26 (1989).

Holdeman, L.V., Good, I.J., and Moore, W.E.: Human fecal flora: variation in bacterial composition within individuals and a possible effect of emotional stress. Appl. Environ. Microbiol. 31, 359-375 (1976).

Hoverstad, T., Fausa, O., Bjorneklett, A., and Bohner, T.: Short-chain fatty acids in the normal human feces. Scand. J. Gastroenterol. 19, 375-381 (1984).

Hudson, M.J. and Marsh P.D.: Carbohydrate metabolism in the colon. In: Human Colonic Bacteria: Role in Nutrition, Physiology, and Pathology (Gibson, G.R. and Macfarlane, G.T., Eds.). CRC Press, Boca Raton, 61-72 (1995).

Hungate, R.E.: Hydrogen as an intermediate in the rumen fermentation. Arch. Mikrobiol. 59, 158-164 (1967).

Lajoie, S.F., Bank, S., Miller, T.L., and Wolin, M.J.: Acetate production from hydrogen and [^{13}C]carbon dioxide by the microflora of human feces. Appl. Environ. Microbiol. 54, 2723-2727 (1988).

Leclerc, M., Bernalier, A., Donadille, G., and Lelait, M.: H_2/CO_2 metabolism in acetogeneic bacteria isolated from the human colon. Anaerobe 3, 307-315 (1997).

Lengeler, J.W., Drews, G., and Schlegel, H.G.: Biology of the Prokaryotes. Blackwell Science, Thieme (1999).

Levitt, M.D., Furne, J., Springfield, J., Suarez, F., and DeMaster, E.: Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. J. Clin. Invest. 104, 1107-1114 (1999).

Levitt, M.D., Springfield, J., Furne, J., Koenig, T., and Suarez, F.: Physiology of sulfide in the rat colon: use of bismuth to assess colonic sulfide production. J. Appl. Physiol. 92, 1655-1660 (2002).

Levine, J., Ellis, C.J., Furne J.K., Springfield, J., and Levitt, M.D.: Fecal hydrogen sulfide production in ulcerative colitis. Am. J. Gastroenterol. 93, 83-87 (1998).

Lewis, S. and Cochrane, S.: Alteration of sulfate and hydrogen metabolism in the human colon by changing intestinal transit rate. Am. J. Gastroenterol. 102, 624-633 (2007).

Ley, R.E., Peterson, D.A., and Gordon, J.I.: Ecology and evolutionary forces shaping microbial diversity in the human intestine. Cell 124, 837-848 (2006).

Lin, C. and Miller, T.L.: Phylogenetic analysis of *Methanobrevibacter* isolated from feces of humans and other animals. Arch. Microbiol. 169, 397-403 (1998).

Lowicka, E. and Beltowsji, J.: Hydrogen sulfide (H_2S)-the third gas of interest for pharmacologists. Pharmacol. Rep. 59, 4-24 (2007).

Macfarlane, G.T., Gibson, G.R., and Cummings, J.H.: Comparison of fermentation reactions in different regions of the human colon. J. Appl. Bacteriol. 72, 57-64 (1992).

Macfarlane, G.T., Hay, S., and Gibson, G.R.: Influence of mucin glycosidase, protease and arylamidase activities of human gut bacteria grown in a 3-stage continuous culture system. J. Appl. Bacteriol. 66, 407-417 (1989).

Macfarlane, S. and Macfarlane, G.T.: Proteolysis and amino acid fermentation. In: Human Colonic Bacteria: Role in Nutrition, Physiology, and Pathology (Gibson, G.R. and Macfarlane, G.T., Eds.). CRC Press, Boca Raton, 75-94 (1995).

Magee, E.A., Richardson, C.J., Hughes, R., and Cummings J.H.: Contribution of dietary protein to sulfide production in the large intestine: An *in vitro* and a controlled feeding study in humans. Am. J. Clin. Nutr. 72, 1488-1494 (2000).

Martens, C.S. and Berner, R.A.: Methane production in the interstitial waters of sulfate-depleted marine sediments. Science 185, 1167-1169 (1974).

Mandar, R. and Mikelsaar, M. Transmission of mother's microflora to the newborn at birth. Biol. Neonate. 69, 30-35 (1996).

Mayer, R., Wong, W.D., Rothenberger, D.A., Goldberg, S.M., and Madoff, R.D.: Colorectal cancer in inflammatory bowel disease: a continuing problem. Dis. Colon. Rectum 42, 343-347 (1999).

McInerney, M.J. and Bryant, M.R.: Anaerobic degradation of lactate by syntrophic associations of *Methanosarcina barkeri* and *Desulfovibrio species* and effect of H_2 on acetate degradation. Appl. Environ. Microbiol. 41, 346-354 (1981).

Miller, T. L. and Wolin, M.J.: Fermentations by saccharolytic intestinal bacteria. Am. J. Clin. Nutr. 32, 424-429 (1979).

Miller, T. L. and Wolin M. J.: Enumeration of *Methanobrevibacter smithii* in human feces. Arch. Microbiol. 131, 14-18 (1982).

Miller, T.L., Weaver, G.A., and Wolin, M.J.: Methanogens and anaerobes in a colon segment isolated from the normal fecal stream. Appl. Environ. Microbiol. 48, 449-450 (1984).

Miller, T.L. and Wolin, M.J.: Stability of *Methanobrevibacter smithii* populations in the microbial flora excreted from the human large bowel. Appl. Environ. Microbiol. 45, 317-318 (1983).

Moore, J., Babidge, W., Millard, S., and Roediger, W.: Colonic luminal hydrogen sulfide is not elevated in ulcerative colitis. Dig. Dis. Sci. 43, 162-165 (1998).

Moore, W.E. and Holdeman, L.V.: Human fecal flora: The normal flora of 20 Japanese-Hawaiians. Appl. Microbiol. 27, 961-979 (1974).

Morris, M.E. and Murer, J.: Molecular mechanisms in renal and intestinal sulfate (re)absorption. J. Membr. Biol. 181, 1-9 (2001).

O'Keefe, S.J.D., Kidd, M., Espitalier-Noel, G., and Owira, P.: Rarity of colon cancer in Africans is associated with low animal product consumption, not fiber. Am. J. Gastroenterol. 94, 1373-1380 (1999).

O'Keefe, S.J.D., Chung, D., Mahmoud, N., Sepulveda, A.R., Manafe, M., Arch, J., Adada, H., and van der Merwe, T.: Why do African Americans get more colon cancer than Native Africans? J. Nutr. 137, 175S-182S (2007).

O'Keefe, S.J.D., Ndaba, N., and Woodward, A.: Relationship between nutritional status, dietary intake patterns and plasma lipoprotein concentrations in rural black South Africans. Hum. Nutr. Clin. Nutr. 39, 335-341 (1985).

Onderdonk, A.B., Hermos, J.A., Dzink, J.L., and Bartlett, J.G.: Protective effect of metronidazole in experimental ulcerative colitis. Gastroenterology 74, 521-526 (1978).

Onderdonk, A.B., Hermos, J.A., and Bartlett, J.G.: The role of the intestinal microflora in experimental colitis. Am. J. Clin. Nutr. 30, 1819-1825 (1977).

Oremland, R.S. and Taylor, B.F.: Sulfate reduction and methanogenesis in marine sediments. Geochim. Cosmochim. Acta 42, 209-214 (1978).

O'Shaughnessy, J.A., Kelloff, G.J., Gordon, G.B., Dannenberg, A.J., Hong, W.K., Fabian, C.J., Sigman, C.C., Bertagnolli, M.M., Stratton, S.P., Lam, S., Nelson, W.G., Meyskens, F.L., Alberts, D.S., Follen, M., Rustgi, A.K., Papadimitrakopoulou, V., Scardino, P.T., Gazdar, A.F., Wattenberg, L.W., Sporn, M.B., Sakr, W.A., Lippman, S.M., and Von Hoff, D.D.: Treatment and prevention of intraepithelial neoplasia: An important target for accelerated new agent development. Clin. Cancer Res. 8, 314-346, (2002).

Park, Y., Hunter, D.J., Spiegelman, D., Bergkvist, L., Berrino, F., van den Brandt, P.A., Buring, J.E., Colditz, G.A., Freudenheim, J.L., Fuchs, C.S., Giovannucci, E., Goldbohm, R.A., Graham, S., Harnack, L., Hartmann, A.M., Jacobs, D.R., Kato, I., Krogh, V., Leitzmann, M.F., McCullough, M.L., Miller, A.B., Peitinen, P., Rohan, T.E., Schatzkin, A., Willett, W.C., Wolk, A., Zeleniuch-Jacquotte, A., Zhang, S.M., and Smith-Warner, S.A.: Dietary fiber intake and risk of colorectal cancer: A pooled analysis of prospective cohort studies. JAMA 294, 2849-2857 (2005).

Parkin, D.M,, Muir, C.S., Whelan, S.L., Gao, J.T., Ferlay, J., and Powell, J.: Cancer incidence in five continents. Lyons (France): International Agency for Research on Cancer, 1992.

Pitcher, M.C.L., Beatty, E.R., and Cummings, J.H.: The contribution of sulphate reducing bacteria and 5-aminosalicyclic acid to fecal

sulphide in patients with ulcerative colitis. Gut 46, 64-72 (2000).

Pitcher, M.C.L., Beatty, E.R., Gibson, G.R., Cummings, J.H.: Incidence and activities of sulphate-reducing bacteria in patients with ulcerative colitis. Gut 36, A63 (1995).

Postgate, J.R. and Campbell, L.L.: Classification of *Desulfovibrio* species, the nonsporulating sulfate-reducing bacteria. Bacteriol. Rev. 30, 732-738 (1966).

Ramasamy, S., Singh, S., Taniere, P., Langman, M.J.S., and Eggo, M.C.: Sulfide-detoxifying enzymes in the human colon are decreased in cancer and upregulated in differentiation. Am. J. Physiol. Gastrointest. Liver Physiol. 291, G288-G296 (2006).

Relman, D.A. and Falkow, S.: The meaning and impact of the human genome sequence for microbiology. Trends Microbiol. 9, 206-208 (2001).

Ridlon, J.M. and Hylemon, P.B.: A potential role for resistant starch fermentation in modulating colonic bacterial metabolism and colon cancer risk. Cancer Biol. Ther. 5, 273-274 (2006).

Roedinger, W.E.: The colonic epithelium in ulcerative colitis: An energy deficiency disease? Lancet 2, 712-715 (1980).

Roedinger, W.E., Lawson, M.J., Kwok, V., Kerr Grant, A., and Pannall, P.R.: Colonic bicarbonate output as a test of disease activity in ulcerative colitis. J. Clin. Pathol. 37, 704-707(1984).

Roediger, W.E., Moore, J., and Babidge, W.: Colonic sulfide in pathogenesis and treatment of ulcerative colitis. Dig. Dis. Sci. 42, 1571-1579 (1997).

Roediger, W.E. and Nance S.: Metabolic induction of experimental colitis by inhibition of fatty acid oxidation. Br. J. Exp. Pathol. 67, 773-782 (1986).

Rose, P., Moore, P.K., Ming, S.H., Nam, O.C., Armstrong, J.S., and Whiteman, M.: Hydrogen sulfide protects colon cancer cells from chemopreventative agent β-phenylethyl isothyocyanate induced apoptosis. World J. Gastroenterol. 11, 3990-3997 (2005).

Savage, D.C.: Microbial ecology of the gastrointestinal tract. Annu. Rev. Microbiol. 31, 107-133 (1977).

Scheppach, W., Fabian, C., Sachs, M., and Kasper, H.: The effect of starch malabsorption on fecal short chain fatty acid excretion in man. Scand J Gastroenterol 23, 755-759 (1988).

Sheppach, W., Sommer, H., Kirchner, T., Paganelli, G.M., Bartram, P., Christl, S., Richter, F., Dusel, G., and Kasper, H.: Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 103, 51-56 (1992).

Segal, I.: Physiological small bowel malabsorption of carbohydrates protects against large bowel diseases in Africans. J. Gastroenterol. Hepatol. 17, 249-252 (2002).

Sitas, F. and Parcella, R.: National Cancer Registry of South Africa. Incidence and geographical distribution of histologically diagnosed cancer in South Africa: South African Cancer Registry Annual Report, Johannesburg. South African Institute of Medical Research, 8-67 (1989).

Sonnenburg, J.L., Chen, C.T.L., and Gordon, J.I.: Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. PLOS Biol. 4, 2213-2226 (2006).

Stolyar, S., Van Dien, S., Hillesland, K.L., Pinel, N., Lie, T.J., Leigh, J.A., and Stahl, D.A.: Metabolic modeling of a mutualistic microbial community. Mol. Sys. Biol. 13, 1-12 (2007).

Tanaka, T., Kohno, H., Suzuki, R., Hata, K., Sugie, S., Niho, N., Sakano, K., Takahashi, M., and Wakabayashi, K.: Dextran sodium sulfate strongly promotes colorectal carcinogenesis in $APC^{Min/+}$ mice: Inflammatory stimuli by dextran sodium sulfate results in development of multiple colonic neoplasms. Int. J. Cancer 118, 25-34 (2006).

Tarrerias, A.L., Millecamps, M., Alloui, A., Beaughard, C., Kemeny, J.L., Bourdu, S., Bommelaer, G., Eschalier, A., Dapoigny, M., and Ardid, D.: Short-chain fatty acid enemas fail to decrease colonic hypersensitivity and inflammation in TNBS-induced

colonic inflammation in rats. Pain 100, 91-97 (2002).

Theissen, U., Hoffmeister, M., Grieshaber, M., and Martin, W.: Single eubacterial origin of eukaryotic sulfide:quinone oxidoreductase, a mitochondrial enzyme conserved from the early evolution of eukaryotes during anoxic and sulfidic times. Mol. Biol. Evol. 20, 1564-1574 (2003).

Toden, S., Bird, A.R., Topping, D.L., and Conlon, M.A.: Resistant starch prevents colonic DNA damage induced by high dietary cooked red meat or casein in rats. Cancer Biol. Ther. 5, 267-272 (2006).

Toden, S., Bird, A.R., Topping, D.L., and Conlon, M.A.: Dose-dependent reduction of dietary protein-induced colonocyte DNA damage by resistant starch in rats correlates more highly with caecal butyrate than with other short chain fatty acids. Cancer Biol. Ther. 6, 253-258 (2007).

Tragnone, A., Valpiani, D., Miglio, F., Elmi, G., Bazzocchi, G., Pipitone, E., and Lanfranchi, GA.: Dietary habits as risk factors for inflammatory bowel disease. Eur. J. Gastroenterol. Hepatol. 7, 47-51 (1995).

Wang, R.: Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter? FASEB J. 16, 1792-1798 (2002).

Wang, R.: The gasotransmitter role of hydrogen sulfide. Antioxid. Redox Signal. 5, 493-501 (2003).

West, B., Lendrum, R., and Walker, G.: Effect of sulphasalazine (salazopyrin) on faecal flora in patients with inflammatory bowel disease. Gut 15, 960-965 (1974).

Whitman, W.B., Coleman, D.C., Wiebe, W.J.: Prokaryotes: the unseen majority. Proc Natl. Acad. Sci. (USA) 95, 6578-6583 (1998).

Widdle, F.: Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In: Biology of Anaerobic Microorganisms (Zehnder, A.J.B., Ed.). John Wiley and Sons, New York. 469-585 (1988).

Willis, C.L., Cummings, J.H., Neale, G., and Gibson, G.R.: Nutritional aspects of dissimilatory sulfate reduction in the human large intestine. Curr. Microbiol. 35, 294-298 (1997).

Wilson, E.O.: Consilience: The unity of knowledge. Vintage (1999).

Wilson, M.: Microbial Inhabitants of Humans. Cambridge University Press, Cambridge (2005).

Willemsen, L.E., Koetsier, M.A., van Deventer, S.J., and van Tol, E.A.: Short chain fatty acids stimulate epithelial mucin 2 expression through differential effects on prostaglandin E(1) and E(2) production by intestinal myofibroblasts. Gut 52, 1442-1447 (2003).

Xu, J., Bjursell, M.K., Himrod, J., Deng, S., Carmichael, L.K., Chiang, H.C., Hooper, L.V., and Gordon, J.I.: A genomic view of the human-*Bacteroides thetaiotaomicron* symbiosis. Science 299 (5615), 2074-2076 (2003).

Zhao, W., Zhang, J., Lu, Y. and Wang, R.: The vasorelaxant effect of H_2S as a novel endogenous gaseous K_{ATP} channel opener. EMBO J. 20, 6008-6016 (2001).

Zhi, L., Ang, A.D., Zhang, H., Moore, P.K., and Bhatia, M.: Hydrogen sulfide induces the synthesis of proinflammatory cytokines in human monocyte cell line U937 via the ERK-NF-kappa ß pathway. J. Leukoc. Biol. 81, 1322-1332 (2007).