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Protein Degradation in the Large Intestine: Relevance to Colorectal Cancer

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Abstract

Colorectal cancer is the second most common form of cancer death in Western countries. Diet has been implicated in the aetiology of this disease. Epidemiological evidence suggests that diets high in meat and fat and low in fermentable carbohydrate increase colorectal cancer risk. One mechanism that could explain the association with meat is increased colonic protein metabolism due to increased protein intake from high meat diets. Products of colonic protein degradation and metabolism include ammonia, phenols, indoles and amines which have been shown to exert toxic effects *in vitro* and in animal models. These compounds are present in faecal samples suggesting that they may exert gut mucosal effects. Human studies have shown that colonic protein metabolism via the gut microflora is responsive to dietary protein as faecal ammonia and urinary phenolic compound concentrations increase in response to increased intake of protein rich foods. Other toxic metabolites from dietary protein precursors such as *N*-nitroso compounds and sulphides are also formed. Recent work has shown that diets high in meat, fat and low in fibre increase human faecal water genotoxicity. It is likely that metabolites from colonic protein metabolism contribute to this increase in genotoxicity during high meat intakes.

Introduction

Colorectal cancer is the second most common cause of death from cancer in Western countries (Potter, 1996). In high incidence populations, the majority of colorectal cancer cases tend to be sporadic hence implying a role for environmental factors. Most specifically, diet is thought to be an important factor as 80% of colorectal cancer cases have been attributed to dietary factors (Willett, 1995). Evidence from epidemiological studies show high rates of colorectal cancer in populations consuming diets high in meat and fat and low in starch, NSP (non-starch polysaccharides, fibre) and vegetables. In general, prospective studies tend to support these findings although estimates of relative risk are not high (Bingham, 2000). In 1995, Potter reported that approximately 30 case-control and cohort studies had been carried out to explore the association between cancer risk and meat, fat or protein consumption. Two thirds of these studies had shown a positive risk for intake of all three dietary factors with very few studies showing an inverse association. Prospective studies showing a positive association with protein refer to protein from red meat sources (Table 1). Following review of the epidemiological literature, two recent reports stated

Table 1. Relationship Between Dietary Protein Sources and Colorectal Cancer Incidence as Shown by Prospective Studies

Reference	Study Population	Evidence
Stemmermen *et al*, 1984	7074 men of Japanese ancestry aged 45-68 years	Protein (%calories) (↓)*
Phillips and Snowdon, 1985	25,493 Seventh Day Adventists	Meat (0) Eggs (↑)
Willett *et al*, 1990	88,751 women aged 34-59 years	Fresh red meat (↑)* Processed meat (↑)* Poultry and fish (↓)*
Thun *et al*, 1992	764,343 adults	Red meat (0)
Goldbohm *et al*, 1994	120,852 aged 55-69	Fresh red meat (0) Processed meat (↑)* Poultry and fish (↓)
Giovannucci *et al*, 1994	47,949 male health professionals aged 40-75	Fresh red meat (↑)* Processed meat (↑)* Poultry and fish (↓)* Non red meat protein(↓)*
Gaard *et al*, 1996	50,535 aged 20-24	Processed meat (↑)
Hsing *et al*, 1998	17, 633 males aged >35	Red meat (↑)
Fraser, 1999	34,192 Seventh day Adventists	Red meat (↑)

(↑) = positive association; (↓) = negative association; (0) = no association
* statistically significant association.

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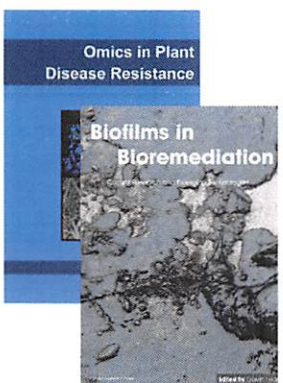

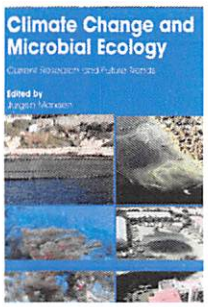

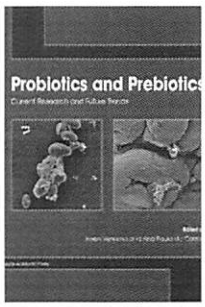

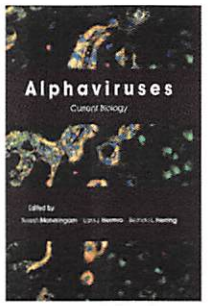

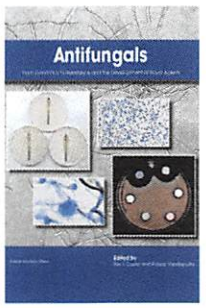

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that red meat probably increases colorectal cancer risk. No association with white meat or fish was apparent due to insufficient evidence (WCRF, 1997; Department of Health, 1998).

Mechanisms whereby red meat may be involved in colorectal carcinogenesis are unknown. Evidence from animal work so far does not suggest a role for promotion by meat (Parnaud *et al.*, 1998). There are animal studies however which suggest a role for cooked meat containing high levels of heterocyclic amines (HAA) (Layton *et al.*, 1995; Pence *et al.*, 1998). Some of these compounds have been shown to be carcinogenic in the large gut and are formed from amino acids during cooking at a high temperature (Skog *et al.*, 1995). Formation of HAA upon cooking meat may be one mechanism to explain the epidemiological association between colorectal cancer risk and meat intake. Other hypotheses involve saturated fat and undigested protein which is metabolised in the colon forming a number of compounds which are known to exert toxic effects. The following review discusses the contribution of the gut flora to this metabolism and possible toxic effects to the host.

Protein Degradation and Metabolism in the Large Intestine

The human large intestine is host to a diverse range of bacteria with total numbers reaching 10^{11} to10^{12} CFU per ml of faecal material (Parodi, 1999). The composition of the gut microflora varies between individuals (Drasar, 1976). So far, diet is known to exert only a modest influence on the flora composition (Hill, 1981) although future advances in identification methodology may provide more information. The principal role of the gut microflora is to salvage energy from non-digestible dietary substrates and endogenous mucus during fermentation. Carbohydrates and protein are the main fermentative substrates in the large intestine (MacFarlane and Cummings, 1991). The main products of this metabolism include gases, hydrogen, carbon-dioxide, short-chain fatty acids (SCFA), branched chain fatty acids, lactic acid, ethanol, ammonia, amines, phenols and indoles (Roberfroid *et al.*, 1995). SCFA especially butyrate are important sources of energy for colonocytes (MacFarlane and Cummings, 1991) and the cancer protective effects of butyrate have been reviewed

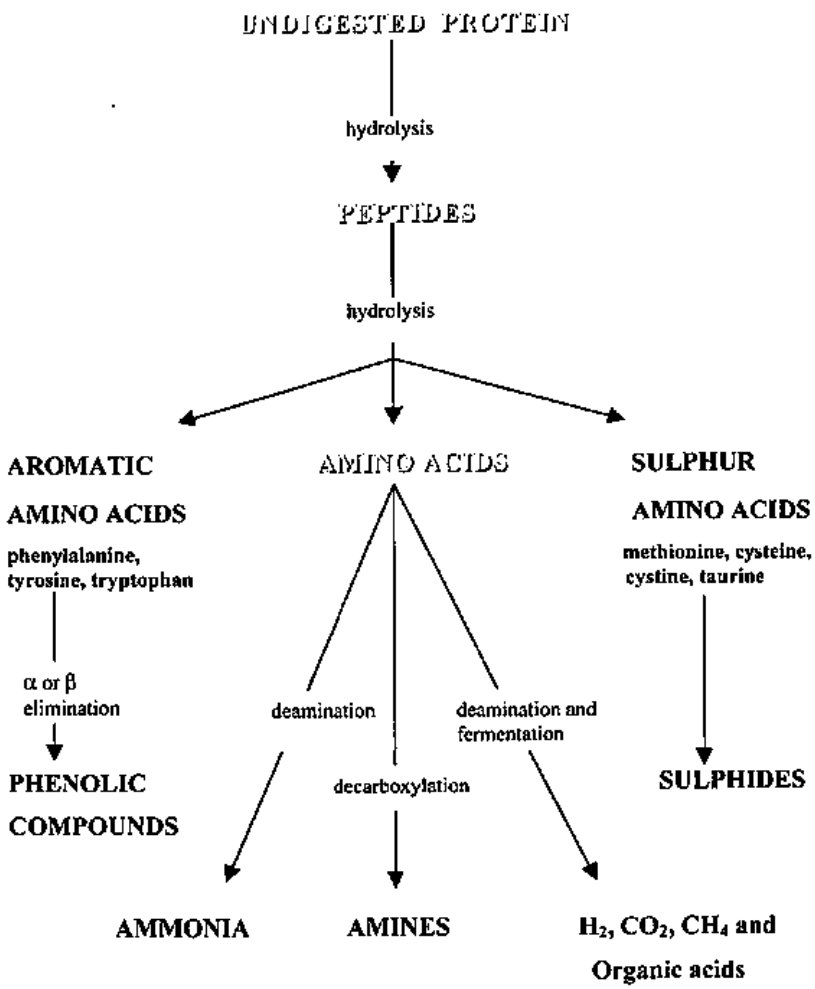

Figure 1. Colonic protein metabolism.

elsewhere (Smith *et al.*, 1998). The nature and extent of fermentation depends upon the characteristics of the bacterial microflora, colonic transit time and the availability of nutrients. The products of carbohydrate metabolism are thought to benefit the host as compared to the toxic end products of protein metabolism. The beneficial effects of high fibre diets in the colon have been previously reviewed (Bingham, 1990 and 1996; Slavin *et al.*, 1997).

The large intestine has been described as a site of intense protein turnover (Macfarlane *et al.*, 1986). Numerically important proteolytic species identified in the large bowel include species belonging to the genera *Bacteroides, Propionibacterium, Clostridium, Fusobacterium, Streptococcus* and *Lactobacillus* (MacFarlane and Cummings, 1991). Protease enzymes of bacterial origin may be extracellular or cell bound and those detected in human faecal samples include trypsin, chymotrypsin, elastase, serine, cysteine and metalloproteinases (MacFarlane *et al.*, 1988; Gibson *et al.*, 1989). On average, 12g of proteinaceous material or 0.5 - 4g total nitrogen, enters the large intestine each day mainly in the form of protein (48 - 51%) and peptides (20 - 30%). Dietary sources make up at least 50% of this protein material, however the amount may vary due to protein intake and the physical form of the food (Chacko and Cummings, 1988; Silvester and Cummings, 1995). In humans, dietary nitrogen is positively associated with nitrogen detected in ileal effluent ($p = 0.005$) (Silvester and Cummings, 1995). This finding shows that it is the amount of protein in the diet rather than its source that determines the amount reaching the colon. The remaining proteinaceous material arriving at the colon is made up of endogenous material, namely pancreatic enzymes, mucus and exfoliated epithelial cells (Chacko and Cummings, 1988). In the large intestine, nitrogenous residues are initially depolymerized by a mixture of residual pancreatic endopeptidases and bacterial proteases and peptidases (MacFarlane *et al.*, 1988) forming short peptides and amino acids available for fermentation. Carbohydrate fermentation products are also formed during protein fermentation i.e. SCFA, hydrogen, CO_2 and biomass, in addition to branched chain fatty acids such as isobutyrate, isovalerate and 2-methylbutyrate, together with other organic acids. Ammonia, amines, phenols and indoles are also formed following deamination, decarboxylation, fermentation and α or β elimination reactions (Figure 1). Concentrations of protein fermentation products are higher in the left sided (distal) colon as compared to the right sided (proximal) colon suggesting that protein fermentation is more prevalent in the distal colon. Once carbohydrate sources are exhausted in the proximal colon, sources of protein material are fermented and metabolised to salvage energy (Gibson, 1996). Although proteins provide a less significant energy source in the large intestine their importance lies mainly in the effects they have on intermediary metabolism of the host and their role as potential systemic toxins.

Products of Colonic Protein Degradation and Metabolism

Ammonia

Amino acid deamination is the most important source of ammonia in the large intestine (Wrong *et al.*, 1985). Ammonia concentrations detected in human faeces range from 12 to 30 mM and excretion has been shown to increase with increased protein intake (Cummings *et al.*, 1979; Silvester *et al.*, 1997; Geypens *et al.*, 1997, Hughes, 1999). Fermentable carbohydrates have been shown to decrease ammonia *in vitro* (Vince *et al.*, 1978; Mortensen *et al.*, 1991; Silvester *et al.*, 1995) and faecal ammonia excretion in humans at low protein intakes (Kelsay *et al.*, 1978). Bacteria assimilate ammonia to form bacterial protein during carbohydrate fermentation, so the concentration of ammonia in the colon at any one time depends upon the balance between amino acid deamination and bacterial protein synthesis. Ammonia exhibits a number of effects that suggest that it may be involved in tumour promotion. Concentrations as low as 5 - 10 mM have been shown to alter the morphology and intermediary metabolism of intestinal cells, affect DNA synthesis and reduce the lifespan of cells (Visek, 1978). Moreover, it has been shown to increase the incidence of colon carcinomas induced by *N*-methyl-*N*-nitro-*N*-nitrosoguanidine in rats (Clinton *et al.*, 1988). Uterosigmoidoscopy patients who have a luminal ammonia concentration as high as 100 mM have increased risk of developing tumours distal to the site of ureteric implantation (McConnel *et al.*, 1979; Tank *et al.*, 1973). Normally ammonia is rapidly absorbed into the portal blood, converted to urea in the liver and excreted in the urine. This pathway is interrupted in cases of liver disease resulting in an accumulation of ammonia in body fluids which is associated with hepatic coma (portal systemic encephalopathy) (Weber *et al.*, 1987).

Phenolic Compounds

Phenolic compounds are formed following bacterial degradation of the aromatic amino acids phenylalanine, tyrosine and tryptophan. Degradation products include p-cresol and phenylpropionate (from tyrosine), phenylacetate (from phenylalanine) and indole propionate and indole acetate (from tryptophan). Intestinal bacteria involved in these processes include *Clostridia* (Elsden *et al.*, 1976) *Bacteroides* (Chung *et al.*, 1975), *Enterobacteria* (Botsford and Desmoss, 1972) *Bifidobacteria* (Aragozzini *et al.*, 1979) and *Lactobacilli* (Yokoyama and Carlson, 1981). Phenolic compounds are absorbed in the colon, detoxified by the liver and excreted in urine principally as p-cresols (> 90% of urinary phenolic compounds) with the remainder being made up of phenol and 4-ethylphenol (Tamm and Villako, 1971). Physiological levels of these compounds in human colonic contents are normally low as bacterial metabolism of aromatic amino acids requires an electron accepting process e.g. nitrate reduction (Young and Rivera, 1985; Bassert *et al.*, 1986). Nevertheless phenolic compounds have been detected in colon contents from sudden death victims and distal concentrations were four times that detected in proximal regions. Simple phenols were the major products of aromatic amino acid metabolism in the

distal bowel supporting the argument that protein metabolism becomes more important in the distal colon as carbohydrate sources are depleted (Smith and MacFarlane, 1996).

Urinary excretion of phenolic compounds is responsive to dietary protein in a positive manner (Cummings *et al.*, 1979; Geypens *et al.*, 1997). In contrast, decreased urinary phenol and cresol excretion has been shown in the presence of readily fermentable carbohydrate (Cummings *et al.*, 1979) and in subjects who changed from a typical Western diet to an uncooked vegan diet (Ling and Hanninen, 1992). Batch culture fermentation studies showed a 60% decrease in the net production of phenolic compounds in the presence of a fermentable carbohydrate (starch) (Smith and MacFarlane, 1996). Like ammonia, it is probable that these nitrogen sources are utilised for bacterial growth when stimulated by carbohydrate fermentation. Longer transit times increased tyrosine and phenylalanine fermentation in an *in vitro* gut model (Smith and MacFarlane, 1996). This suggests that longer retention times in the gut encourage more efficient proteolytic metabolism.

The relation of phenol production to cancer is unclear. *In vitro* work has shown that phenol may enhance *N*-nitrosation of dimethylamine by nitrite and the reaction between phenol and nitrite produces the mutagen diazoquinone (Kikugawa and Kato, 1986).

Amines

Amines found in gut contents include agamatine, tyramine, pyrrolidine, histamine, piperidine, cadaverine, putrescine and 5-hydroxytryptamine (Drasar and Hill, 1974). Species belonging to the genera *Clostridium*, *Bifidobacterium* and *Bacteroides* have been shown to form amines in substantial quantities (Allison and MacFarlane, 1989). Normally, amines produced by colonic bacteria are detoxified by monoamine and diamine oxidases in the gut mucosa and liver. Dimethylamine has been detected in human urine samples and 50% of the levels detected were of bacterial origin (Asatoor and Simenhoff, 1965). Although amines have been linked to migraine, hypertension, hepatic coma (MacFarlane and MacFarlane, 1997) and tyramine from food has been implicated in heart failure (Smith, 1980), the physiological significance to the host is largely unknown. Cancer patients excrete higher levels of N-acetyl and acetoxy derivatives of putrescine and cadaverine as compared to healthy individuals (Murray *et al.*, 1993). Putrescine has been shown to regulate cell growth and differentiation in the gastrointestinal epithelium (Seidel *et al.*, 1984). An emerging area of interest however is in their role as *N*-nitrosation precursors resulting in the formation of potentially carcinogenic *N*-nitrosocompounds as discussed below.

N-nitrosocompounds

Many *N*-nitroso compounds (NOC) are known to exert carcinogenic/mutagenic effects following the formation of potent DNA alkylating agents during metabolism. Preformed NOC are found in cosmetics, pharmaceutical products and occupational sources. However endogenous formation provides the most potent source of exposure for humans (Ohshima and Bartsch, 1981). NOC are formed following the reaction between nitrosating agents and nitrosatable substrates. This reaction may be acid or bacterially catalysed or cell mediated hence *N*-nitrosation may occur at a number of sites in the body (Mirvish, 1995; Tricker, 1997). The large intestine provides a site for bacterially mediated *N*-nitrosation reactions due to the presence of nitrosating agents from dissimilatory nitrate metabolism and nitrogenous residues from endogenous and dietary sources. These nitrosatable substrates include, dietary proteins and peptides, amino acids, secondary amines, indoles and phenols derived from protein metabolism (Shephard *et al.*, 1987) and glycine derivatives such as the bile acid glycocholic acid (Shuker and Margison, 1997). Large intestinal *N*-nitrosation has previously received little attention due to analytical difficulties. The development of a group selective method for total NOC detection however has allowed NOC detection in several biological fluids including faeces (Tricker, 1997). NOC detected by this method are referred to as apparent total NOC (ATNC) as the method may be susceptible to false positives from S-nitrosothiols and nitrolic acids (Walters *et al.*, 1978). Using this approach large intestinal *N*-nitrosation was demonstrated in rats and shown to be dependant upon the presence of a gut microflora (Massey *et al.*, 1988). *In vitro* studies have demonstrated a positive correlation between bacterial nitrate reductase activity and *N*-nitrosation (Calmels *et al.*, 1985, 1988). Denitrifying bacteria are more potent nitrosators than non-denitrifying bacteria following induction under anaerobic conditions in the presence of nitrate or nitrite (Leach *et al.*, 1987). Bacterial strains belonging to *Escherichia*, *Pseudomonas*, *Proteus*, *Klebsiella* and *Neisseria* families have been shown to nitrosate the amine morpholine in the presence of nitrite (Calmels *et al.*, 1985, 1988; Leach *et al.*, 1987). Bacterial *N*-nitrosation was later shown to be dependant upon the presence of nitrate and nitrite reductase genes probably through the production of NO or NO^+ like species (Calmels *et al.*, 1996). Despite evidence from *in vitro* studies the exact mechanism of bacterial nitrosation remains unknown. In terms of colonic nitrosation, the majority of the microorganisms mentioned above are facultative anaerobes and the majority of human large intestinal microorganisms are obligate anaerobes with numbers of facultative anaerobes being many orders of magnitude lower (Gibson, 1996). Nevertheless, ATNC have been detected in faecal samples from healthy human volunteers and excretion is related to dietary nitrate (Rowland *et al.*, 1991) and red meat consumption (Bingham *et al.*, 1996; Silvester *et al.*, 1997; Hughes, 1999). Nitrate and red meat may contribute to large intestinal *N*-nitrosation due to the formation of nitrosating agents from dissimilatory nitrate metabolism and nitrogenous residues from colonic protein degradation.

Table 2 summarises quantitative results from some recent studies. All studies referenced in Table 2 reported a high interindividual variation in faecal ATNC excretion which may be due to individual variations in gut flora composition especially nitrate and nitrite reducing bacteria. In addition recent work has shown that faecal ATNC concentration is positively associated with intestinal transit time and inversely related to faecal output (Hughes, 1999). This

Table 2. Human Studies Showing an Effect of Diet on Faecal ATNC Excretion

Reference	n	Intervention	Mean faecal ATNC
Rowland *et al*, 1991	8	low nitrate	82 μg /kg
	8	300 mg/d nitrate	307 μg /kg‡
Bingham *et al*, 1996	8	60 g/d meat	40 μg/d
	8	600 g/d red meat	113 μg/d*
	8	600 g/d red meat + 20g bran[a]	138 μg/d*
	2	600 g/d white meat	56 μg/g
	2	60 g/d meat	61 μg/d
Silvester *et al*, 1997	8	60 g/d meat	35 μg/d (254 μg/kg)
	8	600 g/d red meat	114 μg/d†(1010 μg/kg)
	8	600 g/d red meat + 37g RS	151 μg/d†(1004 μg/kg)
Hughes, 1999#	8	No meat	51 μg/d (416 μg/kg)
	8	60 g/d red meat	47 μg/d (342 μg/kg)
	8	240 g/d red meat	136 μg/d (1195 μg/kg)#[1]
	8	420 g/d red meat	181 μg/d (1567 μg/kg)#[1]
	11	420 g/d red meat	132 μg/d
	11	420g/d red meat + 400 g/d vegetables[b]	160.9 μg/d

[a] phytate-free wheat bran, RS resistant starch.
[b] vegetables as broccoli, petits pois and brussel sprouts.
‡ significantly different to the low nitrate diet, $p < 0.01$.
* significantly different to the low meat diet, $p < 0.05$.
† significantly different to the 60g/d meat diet, $p < 0.05$.
# $p<0.0001$ for a dose response effect of red meat on faecal ATNC.
#[1] significantly different to the no meat and 60 g/d meat diets, $p < 0.01$.

coincides with previous evidence that longer retention times allow more efficient bacterial proteolytic metabolism (MacFarlane and Cummings, 1991). High stool weights are associated with lower colorectal cancer risk (Cummings et al., 1992) probably due to a dilution effect reducing contact between the colonic mucosa and carcinogenic agents such as NOC. With high meat intakes, fermentable carbohydrate such as phytate-free wheat bran, resistant starch and vegetables, had no effect on faecal ATNC excretion or concentration (Bingham *et al.*, 1996; Silvester *et al.*, 1997; Hughes, 1999). In contrast, Rowland (1996) reported a decrease in faecal ATNC concentration and an increase in total daily output of ATNC as a result of increased intakes of dietary non-starch polysaccharide (NSP). In this study however, a meat-free low residue (Clinifeed) diet was used and substrate availability for ATNC formation may have been limited. The lack of effect of white meat shown by Bingham *et al.*, (1996), complements the epidemiological evidence showing little effect of white meat on colorectal cancer risk. Differences in the effect of red and white meat on faecal ATNC excretion may be explained by dietary haem which is found in higher quantities in red meat. Iron is required for bacterial nitrate reductase activity (Garde *et al.*, 1995) and haem proteins from meat can form nitrosating agents from NO under anaerobic conditions and nitrosate phenol (Wade and Castro, 1990). The sample size in the white meat study however is too small to conclude any effect. Work is underway to investigate the effects of iron on endogenous *N*-nitrosation.

The toxicological significance of increased faecal ATNC excretion is not known as the group selective method for NOC detection gives no information on the individual NOC present. Attempts to characterise the compounds have shown that they are water-soluble, and 50% have a molecular weight less than 3000. Such compounds can cross cell walls and exert effects at the cellular level. Compounds known to be present include acidic and basic nitrosamines which may be genotoxic upon activation by cytochrome P450 enzymes (Silvester *et al.*, 1997).

Sulphur and Sulphur Metabolites

The biology of sulphur in the human gut has escaped serious attention until recently, and thus very little is known of the amounts and sources of sulphur in the diet, and their subsequent digestion and absorption from the intestine. An understanding of the microbial metabolism of sulphur is however well advanced, and in anaerobic ecosystems, like the large intestine, reduced sulphur compounds such as hydrogen sulphide (H_2S), which are highly noxious, can be formed. Emerging evidence suggests sulphide may be toxic to the colonic epithelium. The chief sources of sulphur in the diet are derived from dietary inorganic sulphur (sulphate, sulphite) and the sulphur amino acids, methionine, cysteine, cystine and taurine. Sulphur also occurs naturally in the form of sulphur-containing glucosinolates in brassica vegetables. Intake of inorganic sulphate in humans has been estimated to range between 1.5 and 16 mmol/day (Florin *et al.*, 1991), where dietary sources include fermented beverages, some commercial breads, and dried fruits (Florin *et al.*, 1993). The consumption of sulphur amino acids fluctuates with protein intake. In humans, faecal sulphide concentrations increased from (mean(sem)) 0.22 (0.02) mmol/day to 3.38 (0.31) mmol/day when meat intake increased from 0g/day to 600g/day and this was dose related ($p<0.01$). This result was confirmed by *in vitro* modeling of protein fermentation (Magee *et al.*, in press). In this study the main dietary contribution to protein intake was meat. The effect of other protein sources so far is unknown.

It is difficult to determine the physiological significance of increased faecal sulphide excretion to the host as the exact conditions under which sulphides are toxic to epithelial cells are unknown. Several lines of experimental evidence however implicate sulphide as a damaging agent in inflammatory bowel disease and ulcerative colitis (UC). Perfusion for 4 h of isolated rat colon with 0.2 to 1.0 mmol/L sulphide produced increased mucosal apoptosis and goblet cell depletion (Aslam *et al.*, 1992). Roediger *et al.* (1993a; 1993b) demonstrated inhibition of *n*-butyrate oxidation *in vitro* in both rat and human colonocytes at a concentration of 2 mmol/L. Using human colon tissue, Christl *et al.* (1994) showed that sulfide at 1 mmol/L significantly increased cell proliferation rates and other changes seen classically in ulcerative colitis. Diminished *n*-butyrate oxidation was demonstrated during perfusion of sulfide into the proximal rat colon (Roediger and Nance, 1986; 1990).

The production of sulphide via sulphur amino acid fermentation has been examined *in vitro* in batch culture experiments. Faecal slurries from healthy volunteers were spiked with 10 mmol/L-cysteine resulting in a net sulphide generation of approximately 3 μmol/g/48 h (Florin, 1991). Greater sulphide production has been demonstrated in similar experiments using a methionine spike of 5 mmol/L, resulting in levels as high as 100 μmol/g/24 h (Roediger, 1995). *In vivo*, H_2S, mercaptans and phenols are produced during proteolysis and sulphur amino acid fermentation (MacFarlane and MacFarlane, 1995), predominately in the distal rather than proximal colon (MacFarlane *et al.*, 1992). This observation of regional differences may have implications in the distal distribution of UC. Of the mercapto fatty acids, mercaptoacetate has been shown to occur in concentrations up to 12 mmol/24 h in batch culture experiments of suspensions of human faecal bacteria (Duncan *et al.*, 1990). Thus, good evidence for sulphide production by anaerobic bacteria from sulphur-containing amino acids exists. In addition, it is seems probable that the amounts of dietary inorganic sulphate and sulphur amino acids are critical in determining sulphide production in the large intestine.

Conclusions

Experimental studies have shown that faecal and urinary excretion of protein metabolites (ammonia, phenols and indoles, NOC and sulphides) are elevated as a consequence of increased meat intakes. Colonic protein metabolism may be one mechanism to explain the epidemiological relationship between red meat intake and colorectal cancer risk as certain products of colonic protein degradation such as ammonia, NOC and possibly sulphides, are known to exert toxic effects. Fermentable carbohydrates have been shown to decrease ammonia *in vitro* (Vince *et al.*, 1978; Mortensen *et al.*, 1991; Silvester *et al.*, 1995) and urinary phenol and cresol excretion in humans (Cummings *et al.*, 1979). This may reflect increased carbohydrate metabolism at the expense of protein metabolism as carbohydrate is the energy supplying nutrient favoured by the gut microflora (MacFarlane and Cummings, 1991). There are *in vivo* studies, however, showing no effect of fermentable carbohydrate intake on faecal excretion of ammonia (Cummings *et al.*, 1979; Flourie *et al.*, 1986; Sugawara *et al.*, 1991) and NOC (Bingham *et al.*, 1996; Silvester *et al.*, 1997). In the later studies, fermentable carbohydrate sources were given with a high protein load which may have outweighed the effects of carbohydrate metabolism. Dietary protein intake in relation to fermentable carbohydrate intake may be important when considering the influence of diet on colonic protein metabolites. Faecal measurements reflect metabolite concentrations (typically protein metabolites) in the distal colon, the subsite most prone to disease, more-so than the whole colon. Little is known about colonic NOC and sulphide absorption and the toxicological significance of these compounds to colon health has yet to be elucidated despite known *in vitro* and *in vivo* toxic effects. Recently, diets high in meat and fat and low in dietary fibre have been shown to increase human faecal water genotoxicity (Reiger *et al.*, 1999). Epidemiologically, these diets are associated with increased colorectal cancer risk. It is probable that toxic products from colonic protein metabolism contribute to this increased genotoxicity.

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