

New food components and gastrointestinal health

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Apart from its main functions of digestion, absorption and faecal processing, the human gastrointestinal tract has a complex pattern of muscular activity regulated by a largely autonomous nervous system, and its various organs contain large concentrations of immune and endocrine tissues. Any failure of these closely-integrated systems can lead to diseases ranging from the mildly irritating to the life threatening. Food contains a huge variety of chemical species, many of which are biologically active, and the distal regions of the gut are colonised by a rich and metabolically-active commensal flora that depend on nutrients derived ultimately from the host's dietary residues. The present paper explores the evidence for significant effects of food ingredients on functional bowel disorders, intestinal infections, and aspects of epithelial cell physiology involved in the development of colo-rectal neoplasia. Various strategies, including the manipulation of the colo-rectal microflora with pre- and probiotics, and the development of new products and plant varieties containing biologically-active constituents, have the potential to underpin the development of novel functional food products. However, these products will need to be based on proven biological principles, and fully tested for efficacy and safety. The rapidly-developing fields of functional genomics and cell biology will open up new experimental strategies to explore these possibilities, and emerging processing technologies seem likely to provide novel methods for their exploitation.

Functional foods: Diet: Gut microflora: Irritable bowel syndrome: Colo-rectal cancer

The main functions of the human gastrointestinal tract, i.e. the digestion of food, absorption of nutrients and the fermentation, storage and disposal of dietary residues, require a variety of ancillary processes of which, for most of the time, we remain blissfully unaware. The gut is the largest endocrine organ in the body, it contains most of the body's immune tissues, and its muscular activities are regulated by a complex and largely autonomous nervous system. Complexity does not necessarily imply fragility, but it is certainly true that the gut is a major locus of diseases, ranging from the mildly irritating to the life threatening. Dyspepsia and symptoms of gastro-oesophageal reflux disease are reported by about 30 % of adults, and irritable bowel syndrome (IBS) is currently the most frequent reason for referral to a gastroenterology clinic in the UK. Gastrointestinal infections are amongst the commonest disorders of childhood, and sporadic food poisoning remains a major threat to public health in most Western societies. Later in life, much of the alimentary tract becomes vulnerable to the development of neoplasia; colo-rectal carcinoma, to name but one example, is the second

most common cause of death from cancer amongst men in the UK.

The mucosal tissues of the gut are separated from the lumen by a single layer of columnar epithelial cells specialised for absorption of nutrients, and the exchange of large volumes of water and electrolytes. Much of the susceptibility of the alimentary tract to disease is a direct result of its exposure to food and micro-organisms derived from the external environment. Food contains a huge variety of chemical species, many of which are biologically active, and the distal regions of the gut are colonised by a rich commensal flora that depends on nutrients derived ultimately from our own dietary residues. It would be surprising if the health of the gut were not directly linked to our diets, but our poor understanding of this relationship inhibits the rational development of functional foods for the maintenance of gastrointestinal health. The present paper will briefly explore the evidence for significant effects of food ingredients on examples of functional bowel disorders and intestinal infections, and on some biological processes underlying the development of neoplasia.

Abbreviations: EPA, eicosapentaenoic acid; IBS, irritable bowel syndrome; PUFA, polyunsaturated fatty acids; SCFA, short-chain fatty acids.

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Irritable bowel syndrome

IBS is a common functional disorder of unknown cause. Estimates of its prevalence range up to 20 % of the population at any one time, although such estimates depend on the stringency of the diagnostic criteria used. It is estimated that only about 10 % of those who suffer from IBS symptoms seek medical help (Camilleri & Choi, 1997). The main features of the condition are abdominal pain, sensations of bloating, and a pattern of defecation sufficiently abnormal as to cause distress to the sufferer. About one-third of patients suffer constipation, one-third report diarrhoea, and one-third experience a combination of the two. IBS is a major cause of chronic distress, impaired quality of life and economic cost, but there are few treatment options of proven value. Under these circumstances an effective strategy for dietary intervention would be extremely valuable.

In the early 1970s, Burkitt (1971) observed that African populations consuming traditional rural diets rich in vegetables and cereal foods produced bulkier more-frequent stools than individuals living in the industrialised West. Both functional and degenerative diseases of the gastrointestinal tract are rare in such communities. Burkitt (1971) proposed that the consumption of highly-processed cereals in the West led to chronic constipation and prolonged high pressures within the colonic lumen. Although the more far-reaching versions of the dietary-fibre hypothesis remain unproven, it is certainly true that many individuals consider themselves to be suffering from an unsatisfactory bowel habit, and clinicians have long sought to use bulking agents as a means of treating IBS patients in whom symptoms of constipation predominate.

Recently Akehurst & Kaltenthaler (2001) reviewed forty-five carefully-controlled studies on the treatment of IBS, including seven in which bulking agents classifiable as food ingredients were tested. This analysis provides good evidence that dietary intervention can provide some relief from the symptoms associated with IBS, at least within the setting of a clinical trial, although the evidence for a specific beneficial effect of faecal bulking agents is rather weak. In one typical study (Cook *et al.* 1990) patients consumed four cookies containing 20 g maize fibre or a placebo daily in a double-blind crossover trial for 7 months. There was a statistically significant ($P=0.001$) improvement in symptom scores during both the fibre and placebo treatments, but no evidence of a significant difference between them. One consistent feature of studies with cereal bran is the strong and consistent evidence of a placebo effect associated with the relief of symptoms of IBS (Lucey *et al.* 1987; Snook & Shepherd, 1994). For example, in a dietary intervention study with wheat bran Snook & Shepherd (1994) observed a sustained improvement in reported symptoms in excess of 50 % during the placebo period. Such large effects are consistently observed in the treatment of IBS. They illustrate the difficulty of establishing an objective physiological treatment for functional bowel conditions, but they perhaps also provide an explanation for the commercial success of products that, although they may lack any established mechanism of action, are both enjoyed by consumers and perceived by them as beneficial to gastrointestinal health.

Unlike cereal brans, there is some evidence for a specific beneficial effect of isphagula husk in IBS patients (Prior & Whorwell, 1987). This material is a complex source of dietary fibre, rich in both soluble and insoluble polysaccharides, some of which resist bacterial degradation in the colon. Cellulose gums are already used by the food industry as ingredients to modify the functionality of processed foods, they are highly resistant to fermentation, and they can be modified so as to retain their viscosity in the colon. Cellulose gums are certainly capable of increasing stool bulk and frequency in animal models (Johnson & Gee, 1986), they have been shown to reduce constipation in human subjects (Snape, 1989) and hydroxypropylmethylcellulose has a beneficial effect on plasma cholesterol levels (Dressman *et al.* 1993). Further studies to determine whether they can be used in functional foods designed to regulate stool bulk and frequency in human subjects may, therefore, be appropriate.

Infectious disease

The gastrointestinal lumen is essentially a specialised region of the external environment. The high moisture content, stable temperature and abundant nutrients provide an ideal environment for colonisation by micro-organisms. Bacteria that have become adapted to the digestive tract employ a diverse array of enzymes to exploit food residues as sources of energy. Multicellular organisms commonly take advantage of these enzymes to augment their own digestive capabilities, and human subjects are no exception. In healthy individuals the stomach and small intestine remain relatively sterile, but the large intestine contains about 100 g bacterial cells, representing approximately 400 different species of bacteria. In general, the largest groups present are Gram-negative anaerobes of the genus *Bacteroides*, and Gram-positive organisms comprising a number of different genera including *Bifidobacteria*, *Eubacteria*, *Lactobacilli* and *Clostridia* (Moore & Holdeman, 1974). Bacteria invade the sterile fetal gut at birth by cross contamination from the mother, and then establish themselves more gradually during infancy (Bullen *et al.* 1976). The full microbiological complexity is not achieved until after weaning, when individuals tend to acquire and retain a characteristic microbial pattern. Unfortunately, the nutrient-rich environment of the gastrointestinal tract is also an ideal ecological niche for pathogenic micro-organisms. Infections of the alimentary tract are a major health problem in both developing countries and industrialised societies, and although most gastrointestinal infections are mild and self-limiting, some are intractable and life threatening, and all cause discomfort, inconvenience and considerable socio-economic losses.

Interest in the use of *Lactobacilli* to manipulate the human colonic microflora in order to improve health goes back at least as far as the beginning of the twentieth century. Metchnikoff (1907) proposed that fermented milk could be used as a source of beneficial *Lactobacilli*, and that regular consumption would improve health and prolong life. Modern versions of these ideas continue to stimulate considerable interest amongst consumers and food manufacturers, and they have encouraged various attempts to place the concept on a more rigorous scientific footing. The term

probiotic refers to any live microbial feed supplement that exerts a beneficial effect on the host by modifying the balance of the intestinal microflora (Fuller, 1989). The difficulty with this approach is that live bacteria must traverse the highly acidic environment of the stomach and survive their passage along the small intestine before reaching the colon. To overcome the difficulties posed by competition between the indigenous bacteria and the probiotic organisms, two other approaches have been developed. Prebiotics are 'non-digestible' food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Gibson & Roberfroid, 1995). Prebiotics are usually carbohydrates, ranging in molecular size from sugar alcohols and oligosaccharides to resistant starch (Gibson, 1999). When a probiotic is combined with a prebiotic favouring its growth in the colon it is called a synbiotic (Rolfe, 2000).

Both probiotics and prebiotics are now widely used by the food industry as components of functional foods designed to improve the health of the human digestive system, although the details are often poorly defined. Ultimately, the strength of any claims for such products rests both on their ability to modify the composition of the human colonic microflora, and on their ability to improve some beneficial function or alleviate a recognised clinical symptom or condition. One possibility is that the balance of colonic micro-organisms might be manipulated so as to resist the effects of pathogenic bacteria. For practical reasons, it is extremely difficult to test the ability of probiotics to protect against sporadic gastrointestinal infections in the general population, but well-defined subpopulations with a high risk of pathological changes or infection can be used to test the hypothesis. Two examples are patients undergoing antibiotic therapy, and travellers visiting countries with high levels of infectious gastroenteritis against which they have little natural immunity.

Antibiotic-induced diarrhoea

Diarrhoea is a common side effect of antibiotic therapy, which occurs in about 20 % of patients. Its aetiology is poorly understood, but it probably reflects a gross disturbance of the composition of the colonic microflora, allowing pathogenic forms to proliferate and disrupt colonic fluid absorption and motility. Gotz *et al.* (1979) conducted a double-blind trial in which ninety-eight patients were given a mixture of *Lactobacillus casei* and *Lactobacillus bulgaricus* or a placebo during treatment with ampicillin. Altogether 14 % of the control group experienced antibiotic-induced diarrhoea, compared with none of the treatment group ($P < 0.03$). Surawicz *et al.* (1989) conducted a similar study with 180 patients, using the yeast *Saccharomyces boulardii*. Of the patients in the placebo group 22 % experienced diarrhoea, compared with only 9.5 % of those receiving the live yeast ($P = 0.038$). Although they perhaps have little direct relevance for food manufacturers, these and similar studies establish the principle that micro-organisms, taken as oral supplements, can traverse the upper alimentary tract in a viable state and exert some beneficial effects on the colonic microflora.

Traveller's diarrhoea

Residents of northern industrialised countries travelling to southern latitudes are notoriously susceptible to gastrointestinal infections, often caused by local strains of *Campylobacter*, *Escherichia coli* or rotavirus against which the traveller has a low level of immunity. Although usually mild and self-limiting, such infections cause a high level of inconvenience and loss of effectiveness, and their prevention is highly desirable. There have been a number of studies designed to establish whether probiotic micro-organisms can reduce the risk of traveller's diarrhoea. One of the largest studies reported is that of Oksanen *et al.* (1990) in which 756 travellers from Finland to Turkey received *Lactobacillus GG* as a prophylactic measure, or a placebo, and the outcome was assessed by questionnaire. In total, 43.8 % of the study population reported that they had suffered from diarrhoea, but the incidence was significantly lower ($P < 0.05$) in the treatment group (41.0 %) compared with the placebo group (46.5 %).

These examples seem to provide promising evidence that manipulation of the human colonic microflora has potential as a means of preventing gastrointestinal infections, under circumstances in which a placebo effect seems an unlikely explanation. In this context more recent studies with fructose oligosaccharides which confirm that their use as prebiotics significantly ($P < 0.05$) increases the levels of bifidobacteria in the human colon (Gibson *et al.* 1995; Buddington *et al.* 1996), are particularly relevant, and further research on their potential as prophylactics seems appropriate. To fully assess the significance of both pro- and prebiotics, a much more detailed understanding of the intimate relationship between the colonic microflora and the physiology of the host will be necessary. Our rapidly-expanding knowledge of both human and bacterial genomes, and the increasing use of high-throughput technologies to explore them, will be of enormous value in this area. The recently-published study of Hooper *et al.* (2001), showing a species-specific ability of colonic bacteria to modulate the expression of genes in the intestinal epithelium, is one example of this approach.

Gastrointestinal neoplasia

Colo-rectal carcinoma is currently the second most common cause of death from cancer in many industrialised societies. There are several well-defined hereditary forms of the disease, but these account for no more than 15 % of the total disease burden. The generally-accepted model for the origin and development of the sporadic form of this disease is the adenoma-carcinoma sequence (Winawer, 1999). Adenomatous polyps are common lesions, a small proportion of which become malignant, largely it is thought in response to environmental factors (Hill *et al.* 1978). Populations with a high prevalence of adenomas also have a high incidence of colo-rectal cancer, and individuals who harbour polyps have a much higher risk of developing cancer than individuals with no previous history of polyps. In many Western industrialised countries polyps are very common, and a large proportion of the middle-aged population are affected. Fortunately, the transition from non-invasive adenoma to an

invasive carcinoma is a relatively rare event. A major breakthrough in our understanding of the mechanisms underlying the adenoma–carcinoma sequence came with the discovery that its progression is associated with the acquisition of somatic mutations affecting proto-oncogenes or tumour suppressor genes (Vogelstein *et al.* 1988), and the pattern of mutations depends on the size and rate of growth of the lesion.

The ultimate origin of colonic tumours is thought to lie within the rapidly-dividing mucosal epithelium. The columnar epithelial cells lining the mucosal surfaces of both the small and large intestines are constantly shed into the gut lumen and replaced by new cells emerging from the crypts. In the small bowel the maturing cells migrate to the villi, whilst in the colon, where the mucosa is practically flat, the mature cells occupy gently-ridged inter-cryptal zones. Each crypt is a self-contained proliferating unit. At both sites, cell proliferation is normally at its peak near the base of the crypt, and virtually absent from the uppermost third, nearest the gut lumen. The dividing cells in the basal zone are of two types. The stem cells undergo asymmetric divisions in which one daughter cell retains its physical location and continues to divide, whilst the other type enters a population of dividing transit cells, and undergoes several further symmetrical divisions as it migrates upward from the crypt base. Crypt-cell replication is balanced primarily by exfoliation of senescent colonocytes into the intestinal lumen, but there is also a much smaller component of cell loss resulting from apoptosis in the basal zone of the crypt.

Dietary factors and colo-rectal neoplasia

There is fairly consistent evidence from population studies for an adverse effect of meat consumption on the risk of colo-rectal cancer in North America and Europe (Potter, 2000), and strong evidence also for a protective effect of plant foods (Potter, 1996). Fibre has long been considered one of the most important protective factors in the diet, but many years of research have failed to prove this effect conclusively, or to establish its mechanism of action. However, consistent evidence for protective effects of vegetables has emerged, and coupled with the increased understanding of the molecular events underlying the adenoma–carcinoma sequence, a number of intriguing lines of evidence suggest that a rational mechanistic basis for the development of anti-carcinogenic functional foods is likely to emerge in the next few years. One of the most encouraging findings is that prolonged use of aspirin and other non-steroidal anti-inflammatory drugs can reduce the risk of colo-rectal carcinoma by about 50 % (Lancaster & Silagy, 1994), and cause regression of polyps and earlier precancerous lesions (Takayama *et al.* 1998). At least some proportion of the beneficial effects of non-steroidal anti-inflammatory drugs appears to depend on their ability to induce apoptosis in tumour cells, thus deleting them from the adenoma–carcinoma sequence (Elder *et al.* 2000). The challenge is to identify safe and palatable dietary strategies to achieve similar results.

How might food components influence the initiation and promotion of neoplasia at the cellular level? A number of food-borne colo-rectal carcinogens have been identified,

notably polycyclic aromatic hydrocarbons, heterocyclic aromatic amines (Adamson *et al.* 1996) and *N*-nitroso compounds (Bingham *et al.* 1996). Another possibility is that the mutations associated with the adenoma–carcinoma sequence arise from endogenous causes, and that dietary factors operate primarily on the post-initiation stages of colo-rectal carcinogenesis (Bodmer, 1994). In any case, the rate of crypt cell apoptosis appears to be an important determinant of adenoma–carcinoma progression at every stage of the sequence (Bodmer, 1999). Given the clear evidence that non-steroidal anti-inflammatory drugs both increase apoptosis and suppress the development of colo-rectal neoplasia, what, if any, is the evidence that food-borne factors might act in the same way?

Butyrate

The short-chain fatty acids (SCFA) are the major products of carbohydrate fermentation in both the rumen and the non-ruminant colon; acetate, propionate and butyrate account for about 90 % of SCFA in the human large bowel. Of these three SCFA, butyrate provides about 25 % of the total, and it is usually present at a concentration of approximately 25 mM/kg faecal material in the human caecum (Cummings *et al.* 1987). Butyrate functions as a metabolic substrate for the colonic epithelial cells *in vivo* (Roediger, 1990). Perfusion of the intact colon with a butyrate solution of approximately physiological concentrations stimulates crypt cell proliferation in rats (Kripke *et al.* 1989; Sakata & von Engelhardt, 1983), and instillation of SCFA into the human rectum exerts a trophic effect on the mucosa (Mortensen *et al.* 1991). It has been proposed that two forms of inflammatory bowel disease, diversion colitis and ulcerative colitis, both result from an absence or failure of normal butyrate metabolism, and there is experimental evidence for an anti-inflammatory effect of butyrate enemas in human subjects (Harig *et al.* 1989; Mortensen & Clausen, 1996).

Recently, attention has been focused on the effect of butyrate on tumour cell differentiation, growth kinetics and death. Hague *et al.* (1993, 1995) and other researchers (Heerdt *et al.* 1994) demonstrated that exposure to butyrate at physiologically-relevant concentrations induced apoptosis in human colo-rectal adenoma and carcinoma cell lines. This finding led to the hypothesis that increased intra-colonic butyrate levels associated with colo-rectal fermentation of polysaccharides might favour apoptosis of neoplastic epithelial cells *in vivo*, and hence account for the protective effects of fibre-rich diets (Hague *et al.* 1993). Attempts to test this hypothesis in human subjects have been hampered both by a dearth of suitable surrogate biomarkers for colo-rectal carcinogenesis, and by the difficulty of manipulating the SCFA content of the colo-rectal lumen with any degree of precision. In one study Kashtan *et al.* (1992) set out to explore the effect of soluble fibre derived from oat bran on faecal SCFA and mucosal markers of crypt cell proliferation in a group of volunteers, with or without a history of adenomatous polyps. Two groups were given dietary supplements (16.4 g/d) of either oat bran or wheat bran for 2 weeks. Oat bran is rich in β -glucan, a soluble form of dietary fibre more readily fermentable by colonic micro-organisms than the lignified insoluble polysaccha-

rides of wheat bran (Lund & Johnson, 1991). Consumption of oat bran was associated with a significant decrease in faecal pH, but faecal SCFA and butyrate levels actually fell in the oat-bran group by an amount that approached statistical significance. Apoptosis was not measured, but there was no significant effect of either supplement on the crypt cell labelling index of rectal biopsies obtained before and after the dietary intervention.

In a recent placebo-controlled intervention study (Bonithon-Kopp *et al.* 2000) a dietary supplement of isphagula husk (3.5 g/d) was given to patients with a history of adenomatous polyps to determine the effect on adenoma recurrence. The study was not designed to test any particular mechanistic hypothesis, and no attempt was made to determine either faecal butyrate levels or the rate of apoptosis. The outcome was a modest but statistically significant ($P = 0.042$) increase in the risk of adenoma recurrence in the isphagula-supplemented group. This study probably says little about the effects of fibre-rich diets based on complex foods, but it serves to illustrate the care needed in the development of functional foods designed to modulate crypt cytokinetics. The biological effects of polysaccharides cannot be deduced from a single analytical value for dietary fibre because the physical, chemical and biological characteristics of the various components of fibre vary enormously (Johnson, 1993, 1998). Dietary supplements containing soluble highly-fermentable polysaccharides from unconventional sources need to be treated with caution until more is known about their effects on proliferating crypt epithelial cells in the intact human gut (Wasan & Goodlad, 1996).

Polyunsaturated fatty acids

There is good epidemiological evidence that diets high in polyunsaturated lipids of marine origin are associated with a reduced risk of colo-rectal cancer (Schloss *et al.* 1997), and oral supplementation with purified fish oils rich in the *n*-3 polyunsaturated fatty acids (PUFA) can apparently normalise the spatial distribution of colo-rectal crypt cell mitosis in human subjects (Anti *et al.* 1994). These interesting observations suggest that some forms of *n*-3 PUFA might be beneficial to the colo-rectal mucosa. Triacylglycerols derived from dietary fat are hydrolysed to their component fatty acids during digestion, and then re-esterified before transport and metabolism or storage in adipose tissue. Eicosapentaenoic acid (EPA) and docosahexaenoic acid both function as structural components of cell membranes as well as metabolic substrates.

There is a complex relationship between fat consumption and carcinogenesis in animal models. It has been known since the 1950s that a high fat intake accelerates the development of murine mammary tumours (Tannenbaum & Silverstone, 1953), but more recent studies show that the effect depends on the fatty acid composition of the diet. In general, PUFA of the *n*-6 series, such as linoleic acid, promote the induction of mammary tumours more than saturated fats. Monounsaturated fatty acids are largely without effect, and *n*-3 PUFA are protective (Fay *et al.* 1997). These observations are generally consistent with the observed effects of PUFA on tumour cells *in vitro*. It has been clear for some time that certain essential fatty acids,

notably γ -linolenic acid, arachidonic acid, EPA and docosahexaenoic acid are selectively toxic to tumour cells. Das (1991) demonstrated that the inhibition of tumour cell proliferation in the presence of γ -linolenic acid, arachidonic acid and EPA was caused by selective cytotoxicity, and that cell death was blocked by antioxidants, enhanced by pro-oxidants and proportional to the extent of peroxidation induced in the cells. More recent studies have confirmed these findings, extended them to other cell lines and shown that apoptosis plays an important role in the cytotoxicity induced by PUFA (Finstad *et al.* 1998; Hawkins *et al.* 1998; Ramesh & Das, 1998). In the human colo-rectal adenocarcinoma cell line HT29 incubation with EPA leads first to detachment of the cells from the substratum, followed by apoptosis, which can be enhanced by depletion of intracellular glutathione levels (Latham *et al.* 1998) and blocked with antioxidants and caspase inhibitors (Clarke *et al.* 1999).

Dietary long-chain fatty acids exert important effects on crypt cytokinetics *in vivo*. In the rat, replacement of maize oil with fish oil in a semi-synthetic powdered diet leads to a reduction in crypt cell mitosis (Pell *et al.* 1994). Latham *et al.* (1999) fed rats a semi-synthetic basal diet containing maize oil (80 g/kg) before treatment with the specific colo-rectal carcinogen 1,2-dimethylhydrazine or a sham injection. Immediately after the injections the groups were subdivided; half were transferred to diets in which the maize oil was replaced with fish oil (% (w/w); 97 triacylglycerol, 19 EPA, 8 docosahexaenoic acid) and animals were killed after 24 and 48 h. In the rats fed fish oil the wave of apoptosis induced by treatment with the carcinogen was approximately doubled at both time-points ($P < 0.001$), and crypt cell mitosis was suppressed ($P < 0.05$). These effects were associated with a reduced frequency of precancerous lesions (aberrant crypt foci) after 18 weeks ($P < 0.05$). Clearly, dietary PUFA can modulate crypt cell proliferation and death, and influence the course of induced neoplasia in an animal model, but further studies will be necessary to assess the implications for human health.

Glucosinolate breakdown products

The third example of a food ingredient that can modulate crypt cytokinetics *in vivo* comes from the complex group of food-borne substances known as phytochemicals. The glucosinolates are a group of more than 100 organic S compounds found in both wild and domestic brassica species. Their common structure comprises a β -D-thioglucose group and a sulfonated oxime moiety, bearing a variable side-chain derived from methionine, tryptophan, phenylalanine or one of several branched-chain amino acids (Mithen *et al.* 2000). The glucosinolates themselves are stable and unreactive. They remain compartmentalized within the intact plant cells until tissue damage due to pests, processing or chewing by human subjects brings them into contact with the endogenous enzyme myrosinase. The glucosinolates then undergo rapid hydrolysis, releasing an unstable aglycone that can give rise to a variety of other products, including nitriles and isothiocyanates. These latter compounds are the source of the hot and bitter flavours of mustard (*Brassica nigra*), radishes (*Raphanus sativus*), and a variety of brassica vegetables (Fenwick *et al.* 1983).

The isothiocyanates are currently a focus of intense interest amongst food producers. A number of studies have established their ability to block the mutagenic effects of human carcinogens by modulating the activities of phase I and phase II biotransformation enzymes, including glutathione-S-transferase and UDP-glucuronyl transferase (Hecht 1996, 1999), and a number of patents have been taken out in order to exploit this effect. A high consumption of brassica vegetables is protective against cancers of the lung and alimentary tract (van Poppel *et al.* 1999), and there is growing evidence that consumption of brassica vegetables can modulate the activity of phase II enzyme activity in human subjects (Nijhoff *et al.* 1995a,b; Steinkellner *et al.* 2000). In the case of colo-rectal cancer, the beneficial effects of brassica vegetables may also be partly due to their effects on colo-rectal crypt cell proliferation and apoptosis.

It has been demonstrated that exposure of human cancer cells to benzyl and phenethyl isothiocyanate leads to growth arrest and apoptosis, associated with induction of caspase-3, cleavage of poly(ADP-ribose) polymerase and increased levels of c-Jun N-terminal kinase 1 (Yu *et al.* 1996, 1998). Human leukaemia cells (HL60) and human myeloblastic leukaemia 1 cells have also been reported to undergo caspase-dependent apoptosis following exposure to phenethyl isothiocyanate (Xu & Thornalley, 2000). Musk and colleagues showed that allyl isothiocyanate (Musk & Johnson, 1993), phenethyl isothiocyanate and benzyl isothiocyanate (Musk *et al.* 1995) caused selective growth inhibition and a reduction in clonal survival in the undifferentiated phenotype of the human colo-rectal tumour cell line HT29. In an animal model a diet enriched with the glucosinolate sinigrin suppressed mitosis and induced an increased level of apoptosis in the colo-rectal crypts of rats 48 h after treatment with the colon carcinogen 1,2-dimethylhydrazine, but had no significant effect on crypt cytokinetics in untreated animals (Smith *et al.* 1998). These effects were associated with a reduction in the numbers of aberrant crypt foci induced by 1,2-dimethylhydrazine. These potentially-protective effects were induced by sinigrin at levels well in excess of human exposure, but more recently it has been shown that a juice derived from uncooked Brussels sprout tissue exerted much the same effects as allyl isothiocyanate on HT29 cells *in vitro*, and markedly increased crypt cell apoptosis after treatment with 1,2-dimethylhydrazine in the animal model (Smith *et al.* 2000). It remains to be seen whether glucosinolate breakdown products derived from the diet can suppress the cell cycle and induce apoptosis in the human colo-rectal mucosa.

Conclusion

The human gut is exposed to an almost limitless variety of biologically-active components derived from animal and plant tissues, and from bacterial metabolites. This situation has always been so, and it seems inevitable that the tissues of the alimentary tract are adapted to the presence of some of these constituents to an extent that holds implications for human health. The present brief survey demonstrates that minor food constituents can help to alleviate functional symptoms, and modify human gastrointestinal physiology at the cellular level. The rapidly-developing fields of genomics

and cell biology will open up new ways to explore the implications of these findings, and emerging processing technologies may well provide novel strategies for their exploitation.

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