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Starches, Resistant Starches, the Gut Microflora and Human Health

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Abstract

Starches are important as energy sources for humans and also for their interactions with the gut microflora throughout the digestive tract. Largely, those interactions promote human health. In the mouth, less gelatinised starches may lower risk of cariogenesis. In the large bowel, starches which have escaped small intestinal digestion (resistant starch), together with proteins, other undigested carbohydrates and endogenous secretions are fermented by the resident microflora. The resulting short chain fatty acids contribute substantially to the normal physiological functions of the viscera. Specific types of resistant starch (e.g. the chemically modified starches used in the food industry) may be used to manipulate the gut bacteria and their products (including short chain fatty acids) so as to optimise health. In the upper gut, these starches may assist in the transport of probiotic organisms thus promoting the immune response and suppressing potential pathogens. However, it appears unlikely that current probiotic organisms can be used to modulate large bowel short chain fatty acids in adults although resistant starch and other prebiotics can do so. Suggestions that starch may exacerbate certain conditions (such as ulcerative colitis) through stimulating the growth of certain pathogenic organisms appear to be unfounded. Short chain fatty acids may modulate tissue levels and effects of growth factors in the gut and so modify gut development and risk of serious disease, including colo-rectal cancer. However, information on the relationship between starches and the microflora is relatively sparse and substantial opportunities exist both for basic research and food product development.

Introduction

The early work of Metchnikoff (1907), linking the consumption of live organisms in fermented foods by certain ethnic groups with their greater longevity, carried the implicit assumption that the normal colonisation of the gut was potentially deleterious. The current view is rather different in that the linkage between the microflora is with chronic non-infective diseases and not acute outbreaks of food or water borne infections. Colonic microorganisms have been designated as putrefactive under certain circumstances while other gut bacteria may contribute to

pathologies such as gastritis (McColl 1999) and inflammatory bowel disease (IBD) (Ardizzone *et al.*, 1999). More importantly, the view is emerging that most of the activities and products of the microflora are, in fact, beneficial to the host and they should be promoted. Any deleterious effect is subtle and contributes to ill health of the host through those chronic diseases which these beneficial bacteria help to diminish. The activities of these beneficial organisms can be modified by the substrates which they receive, predominantly through the diet. Some of the most important of these are the starches which contribute substantially to energy intake both as processed and unprocessed cereals, pulses, fruits and vegetables. It is the aim of this review to examine the interactions between the gut microflora and human health and the ways in which starches can influence these relationships.

Gastrointestinal Bacteria and Human Health

The microbial population of the digestive tract is largest in the caecum and proximal colon with fewer numbers and species in the mouth, stomach and small intestine. However, these smaller populations can be important for health outcomes. Oral colonisation (especially by *S. mutans*) varies by individual and is thought to contribute to cariogenesis through leaching of calcium from enamel by organic acids (lactate, acetate) produced by metabolism of dietary carbohydrate (Bibby, 1982). This is a condition of apparently low mortality. Even so, a median estimated cost of dental caries in Australia in 1992 was \$478,000,000 (Crowley *et al.*, 1992), which gives some indication of the costs which can be associated with diet-related disease. Fluoridation of the water supply is an effective mechanism for control and limiting damage (Morgan *et al.*, 1998) but does not attack the primary problem. An effective preventive strategy appears to be oral hygiene plus limiting the supply of substrate to minimise production of organic acids from fermentable carbohydrates at or near the tooth surface.

The relatively sparse flora of the healthy stomach is usually less than 10^3 organisms per gram of contents, comprising chiefly facultative anaerobes such as lactobacilli, staphylococci and streptococci. A specific organism (*Helicobacter pylori*) appears to infect about 60% of the adult population in more affluent countries. Rates of carriage are much higher in some populations which may reflect the possibility that the organism is a zoonosis derived from domestic animals (Dore *et al.*, 1999). *H. pylori* is found adhering to the gastric epithelium and infections with particularly virulent strains are associated with reflux, gastritis, surface ulceration and cancer (Ernst, 1999). The bacterium secretes an enzyme, urease, which liberates free NH_3 from urea. NH_3 is a known cytotoxic agent and carcinogen and it is thought that it (or more accurately, NH_4^+) contributes to the local tissue necrosis, ulceration and, ultimately, cancer (Tsuji *et al.*, 1997). In global terms, gastric carcinoma is a major cause of morbidity and

mortality (World Cancer Research Fund and American Institute for Cancer Research, 1997). Eradication of the bacterium through antibiotic therapy is an obvious option for primary prevention but may not be entirely satisfactory for two reasons. Firstly, a direct connection between infection and gastritis which proceeds through ulceration to gastric carcinoma has yet to be shown. Secondly, it is possible that *H. pylori* may be involved to some degree in the maintenance of normal gastrointestinal motility. Affected individuals in whom the organism has been suppressed or eradicated can develop reflux oesophagitis (Richter *et al.*, 1998). This may be associated with a rise in the incidence of Barrett's oesophagus – believed to predispose to increased risk of oesophageal carcinoma (Heatley and Guillou, 1997). It appears that control, rather than eradication, of *H. pylori* may be a preferred option. In addition, there is evidence implicating *H. pylori* (and other organisms) in the pathogenesis of human cardiovascular disease. Treatment of patients with unstable angina with an antibiotic against *Chlamydia pneumoniae* for 30 days lowers the clinical endpoints for up to 6 months (Gurfinkel *et al.*, 1999). *H. pylori* has been shown to be present in atheromatous plaques (Ellis, 1997). However, a direct causal role for it and other candidate infectious agents in atherogenesis has yet to be proven (Danesh *et al.*, 1998). The gut microflora, however, may influence coronary heart disease (CHD) risk by modulating serum lipid concentrations. Circulating levels of LDL and HDL-cholesterol, which are established risk factors for CHD, appear to be influenced by the population profile and metabolic activity of the colonic microflora (Jenkins *et al.*, 1999).

Rapid peristalsis, and the bactericidal actions of gastric secretions, limit colonisation of the upper small intestine to usually less than 10^5 cells/ml of fluid. Bacterial numbers, especially of the strict anaerobes, increase along the small intestine, attaining a density of between 10^6 - 10^8 /ml in the distal ileum. Small intestinal infections, principally due to food and drinking water contaminated with various microorganisms, are common in developing countries (Farthing 1999). Hypochlorhydria and intestinal motor dysfunction often result in substantial bacterial overgrowth of the small bowel (Brasitus and Sitrin 1990) and illustrate the dynamic nature of the balance between the host and the microflora.

The prime focus for any health-related interaction between gut bacteria and starches is the large bowel. This is the major site of bacterial colonisation (Gibson and Roberfroid, 1995) and fermentation (van Soest, 1995) in the gastrointestinal (GI) system of omnivorous animal species including humans. In adult humans the bacterial population is very large, consisting of about 10^{14} viable cells, which account for about 100 grams or 50% of the wet mass of colonic contents (Cummings and Macfarlane, 1997) and of which about 15 grams of biomass is shed daily in faeces (Cummings and MacFarlane 1991; Hill, 1995). The colonic microflora is taxonomically diverse, consisting of over 50 genera and 400 species (Drasar, 1988; Salminen *et al.*, 1995; Savage, 1986; Mitsuka 1996) in which only a relatively few genera predominate. Bacteria are implicated in the aetiology of several important human large bowel pathologies including colorectal cancer (Rowland *et al.*, 1985; Robertson 1993), irritable bowel syndrome (IBS) (King *et al.*, 1998), and inflammatory bowel

disease (IBD) (Lisby *et al.*, 1994). The nutritional influences on these chronic bowel conditions have been reviewed recently (Topping and Bird, 1999). In westernised countries, large bowel cancer is a serious malignancy in which lifestyle factors, especially diet, are believed to play a causative role in the majority of cases (Szilagyi 1998, Topping and Bird, 1999). Putrefactive species may play a role in the initiation and promotion of carcinogenesis through their ability to metabolise luminal substances to potentially harmful toxins, including mutagens and carcinogens. Various nitrogenous compounds, principally ammonia, amines, indoles and phenols, are products of protein and amino acid degradation by putrefactive bacteria (Robertson 1993) and are considered putative risk factors for neoplastic transformation in the large bowel (Bingham 1999). A specific bacterium, *Streptococcus bovis*, has been implicated in cancer of the large bowel (Macaluso *et al.*, 1998). This organism has been shown to colonise large bowel carcinomas, adenomas and polyps but recent studies indicate that the carriage rate of *S. bovis* in patients with confirmed colorectal neoplasms is low (Norfleet and Mitchell 1993) and not significantly different from controls (Potter *et al.*, 1998). Overall, there is little evidence supporting a direct role for this or other bacterial agents in initiating or promoting colorectal cancer. The same appears to be true for the aetiology of constipation and diverticular disease which are manifest as inadequate defaecation and, in the case of the latter, by herniation of the colon. These conditions relate principally to a sub-optimal fibre intake and can be prevented and managed by increasing fibre consumption (Topping and Bird, 1999). IBS is characterised by disordered gut motility and pain with bloating, diarrhoea and/or constipation and while it can affect the whole gut, its primary focus appears to be the large bowel. The condition appears to be unrelated to fibre intake. IBD occurs in two main forms – ulcerative colitis (UC) and Crohn's disease. The former occurs mostly in the distal colon and is characterised by surface ulceration and bleeding. Crohn's disease may be found throughout the GI tract but has a primary focus at the ileo-caecal junction. The microflora have been implicated in both conditions but it appears that disordered cytokine production (possibly due to inadequate *n-3* fatty acid intake) is involved in Crohn's disease (Topping and Bird, 1999).

The composition and state of the intracolonic environment is largely a product of the metabolic actions of the microflora and their interactions with their nutrient supply. Generally, these are viewed as positive and occur through the production of organic acids, especially short chain fatty acids (SCFA). These acids are important regulators of colonic physiological processes and appear essential for maintaining normal bowel function (Cummings and Macfarlane 1991; Velazquez *et al.*, 1996).

Large Bowel Microflora - Substrates and Carbohydrate Fermentation

Supply of Fermentable Substrates

Normally the bacteria which ferment carbohydrates escaping small intestinal digestion and also any undigested proteins of exogenous or endogenous origin predominate in the large bowel ecosystem. This bacterial profile reflects the supply of fermentable substrates which reach the large bowel (Table 1). Of dietary plant polysaccharides and

Table 1. Potential Bacterial Substrates of Dietary Origin Reaching the Colon in Adult Humans Consuming a Westernised Diet

Substrate	Amount (g/day)
Resistant Starch	8-40
Non Starch Polysaccharides	8-18
Oligosaccharides	2-8
Simple Sugars	2-10
Proteins	3-9

From Cummings and Macfarlane, 1991; Baghurst *et al.*, 1996.
Note that endogenous secretions (6-9 g/day) and sloughed intestinal cells (unquantified) also contribute substrates for the colonic microflora.

oligosaccharides, humans possess intrinsic hydrolytic enzymes to digest only starch and its breakdown products. All other non-starch polysaccharides (NSP, major components of dietary fibre) and oligosaccharides (OS) such as fructo-oligosaccharides (FOS), are not digested in the small intestine and enter the large bowel. Depending on age and genetic factors, a variable fraction of lactose may be digested in the small intestine. Monosaccharides (*e.g.* fructose and sorbitol) which are absorbed incompletely in the small intestine also may be fermented. A variable, but substantial, fraction of dietary starch (resistant starch, RS) also enters the human large bowel where it is fermented (Annisson and Topping, 1994; Asp *et al.*, 1996). RS may be an important substrate for the human colonic microflora and its fermentation may convey substantial benefits to the host (Cummings *et al.* 1996).

The passage of NSP into the colon helps to explain the enhanced faecal bulking and promotion of laxation which is one of the best-documented effects of fibre-rich foods such wheat bran (Topping and Bird, 1999). Nevertheless, faecal excretion of NSP is generally incomplete as a fraction is fermented by the microflora (Stephen and Cummings, 1981). This enables bacteria to proliferate with the nitrogen requirement being met by protein and peptide fermentation and, to a lesser extent, by urea. Thus, the voided stool is a composite of undigested dietary components, endogenous secretions as well as bacteria. The degree of susceptibility of carbohydrates to bacterial digestion is termed "fermentability" and varies between foods. Wheat bran is of relatively low fermentability (<50%) while fruit and vegetable fibres appear to be more susceptible to fermentation (Stephen and Cummings, 1981). There appears to be an important gender difference in that transit is slower and fermentability greater in women than men (Stephen *et al.*, 1986; Lampe *et al.*, 1993). Whether this difference extends to the bacterial population is uncertain. If it does, then it raises important issues including the age at which any difference becomes established.

It was assumed that for both sexes, NSP were the principal substrates for fermentation and that ileal starch digestibility was complete as little appears in human faeces under normal circumstances. This was despite indirect evidence (*e.g.* from breath H₂ evolution) that there was escape of starch into the colon (Anderson *et al.*, 1981). However, when it became clear that NSP alone could not meet the substrate requirements of the microflora – the "carbohydrate gap" – RS became a candidate to meet the deficit (Stephen, 1991). It had been shown by direct intubation of the human small intestine that RS could

contribute to large bowel fermentation (Stephen *et al.*, 1983). Other carbohydrates, such as oligosaccharides derived from wheat, onions, endive and similar sources (Moshfegh *et al.*, 1999) not measured commonly in food analysis may make a smaller contribution to filling this gap. However, RS is thought to be the largest contributor to the missing carbohydrate and hence to SCFA production.

As in the rumen or large bowel of obligate herbivores, human large bowel bacteria hydrolyse complex carbohydrates to their constituent monosaccharides. These are then metabolised to the major end products providing energy for bacterial growth. In addition to heat of fermentation and gases (CO₂, H₂ and CH₄), the main metabolic products are SCFA which, in normal adults, are principally acetate, propionate and butyrate (Cummings and Macfarlane, 1991). These acids are the principal anions in normal large bowel digesta and faeces. Generally, they are found in gut contents at concentrations in excess of 80 mmol/L and are thought to play a substantial role in maintaining colonic integrity.

Factors Influencing Starch Digestion

Potentially, starches may be hydrolysed completely to glucose by human small intestinal α -amylase and associated enzymes. The degree of hydrolysis is raised considerably by cooking with water (gelatinisation). Heating with restricted moisture may effect a limited internal rearrangement of the starch polymers in the granule which may increase their resistance to amylolysis. Various factors, including physical inaccessibility of the starch in the food, and its granular structure and degree of retrogradation can limit ileal starch digestibility to less than 100% (Annisson and Topping, 1994; Asp *et al.*, 1996). Chemical modifications (etherisation, esterification and cross-bonding) used by the food industry to produce starches with desirable food technological properties also lower ileal starch digestibility (Brown *et al.*, 1995). The classification of RS into four main types reflects these influences (Table 2) and the RS content of a food can vary with each classification according to the nature and type of components in the food, food manufacturing processes and conditions, storage and how the food is consumed. Obviously, for RS1 the degree of milling can be a prime determinant of digestibility. Starch is found in two main forms, amylose and amylopectin (Annisson and Topping, 1994; Baghurst *et al.*, 1996). The former is a relatively small (100-10,000 monomer units), essentially linear, polymer in which glucose units are linked by α (1 \rightarrow 4) linkages (Shannon and Garwood, 1984). In contrast, amylopectin is an extremely large (normally 10,000-100,000 monomer

Table 2. Nutritional Classification of Resistant Starches

Types of Resistant Starch	Examples of Occurrence
RS1-Physically inaccessible	Whole or partly milled grains and seeds
RS2-Resistant granules	Raw potato, green banana, some legumes and high amylose starches
RS3-Retrograded	Cooked and cooled potato, bread and cornflakes
RS4-Chemically modified	Etherised, esterified or cross-bonded starches (used in processed foods)

units) glucose polymer containing many α (1 \rightarrow 6) branch points. The positions of the branch points relative to the linear regions of amylopectin dictate its cluster and structural properties in the crystalline region of the starch granule. The majority of the amylose exists in the amorphous regions of the starch granules. Most starches available commercially contain at least 70-80% of the total as amylopectin which means that they gelatinise relatively easily on heating with water. Starches from specific maize cultivars can contain much more amylose. Those available currently contain approximately 70% of the total as amylose but newer maize cultivars having >80% of starch as amylose are appearing. The amylose:amylopectin ratio is significant for RS2 and RS3. All high amylose maize starches have a structure and conformation which elevates gelatinisation temperatures compared with regular maize starch. However, once gelatinised, the high proportion of amylose leads to rapid retrogradation of the starch paste (Brown *et al.*, 1998). Retrogradation is a phenomenon which occurs on cooling (*e.g.* potato salad) and involves reassociation of the linear regions of the polymers to form insoluble crystallites which resist enzymatic hydrolysis (Sievert and Pomerantz, 1989). These consist of small amylose domains between amylopectin regions in the cooled cooked starch (Gidley *et al.*, 1995). The RS4 content of a food can be affected by the degree of substitution (Wurzburg, 1986) which means that processed foods containing modified starches can vary in RS content on both this variable and the absolute degree of incorporation. Normally these starches are limited to degrees of substitution which are quite low (<2.5% for acetylated starch). In addition to these reasonably well defined factors, there are physiological influences on RS which can be equally strong but also hard to quantify. For example, large particles (*e.g.* those which have been chewed relatively little) move through the gut more quickly than small ones (Lewis and Heaton 1999; McIntyre *et al.*, 1997). Thus, starch in a small food particle may be hydrolysed quite rapidly compared with that in a large particle. If the transit time from mouth to ileocaecal valve were shorter for the former, then its effective RS content could be quite high compared with the more rapidly digested starch. Obviously, chewing and transit vary by individual and are hard to incorporate into a purely chemical determination of RS which rely principally on the *in vitro* incubation of starches with amylase and/or amyloglucosidase (Englyst *et al.*, 1996). These personal influences have led to the description of RS as "physiological" and "chemical" RS (Annison and Topping, 1994). The former term embraces RS as it occurs in the body and the latter the values obtained by chemical analysis. The differences between physiological and chemical RS may be considerable for specific foods. For example, baked beans or brown rice contain low amounts of RS when analysed by standard procedures (Cheng and Yu 1997; Parchure and Kulkarni 1997). However, in animals the contribution of starch escaping into the large bowel is enough to double the effective "fibre" content of these foods (measured as digesta mass or SCFA) when compared with high fibre/low starch foods such as wheat or rice bran at the same level of dietary fibre (Topping *et al.*, 1993; Marsono *et al.*, 1993). Muir and colleagues (1993) and Åkerberg *et al.*, (1998) have attempted to

replicate physiological influences, specifically chewing, in RS analysis and their data suggest that it is an important factor. This means that it is quite difficult to give an exact figure for a particular person's RS intake. Generally, it is assumed that about 10% of ingested starch may enter the colon (Baghurst *et al.*, 1996).

SCFA and the Large Bowel

Effects of SCFA may be subdivided into the general and specific. The former relate substantially to the fact that the major SCFA are organic acids with pK_a values of approximately 5 so that their production leads to acidification of the intracolonic environment. It is a common finding that promotion of SCFA production through provision of additional fermentable carbohydrate gives lower pH values in the large bowel contents of animals (*e.g.* Topping *et al.*, 1993) and in faecal samples of humans (*e.g.* Noakes *et al.*, 1996) compared with diets low in fermentable carbohydrate. Greater acidity inhibits the growth of potentially pathogenic pH-sensitive organisms (Prohaszka *et al.*, 1990). Lowering of pH leads also to the dissociation of alkaline compounds with toxic or carcinogenic potential and so inhibits their absorption. Other effects which appear to be common to the major SCFA include the stimulation of colonic blood flow (through the dilation of resistance blood vessels) and enhancement of colonic muscular contraction (for more extensive reviews see Cummings *et al.*, 1995). These changes raise muscular tone and large bowel oxygenation and nutrient transport (Kvietys and Granger, 1981). Uptake of SCFA by the large bowel is accompanied by that of fluid and electrolytes. It was thought that this was limited to K^+ and Na^+ but now it seems likely that substantial uptake of Ca^{2+} occurs also and that this may be enhanced by greater provision of fermentative substrate such as OS (Coudray *et al.*, 1997). SCFA promote colonocyte proliferation and thereby assist in reversing the colonic atrophy which is a feature of low-fibre diets. SCFA also maintain a normal phenotype in this population of frequently replaced cells. *In vitro* data indicate that SCFA are important metabolic fuels for colonocytes with butyrate being their preferred substrate (Roediger 1982) and that their capacity to oxidise butyrate is modulated by the microflora (Cherbuy *et al.*, 1995). Cell culture studies show that the presence of butyrate at physiological concentrations enhances growth of normal cells and inhibits that of malignant ones. These actions are effected by a variety of mechanisms including promotion of DNA repair and differentiation of tumour cells (Smith *et al.*, 1998). One important action of butyrate is to induce apoptosis in malignant cells (Smith *et al.*, 1998). In normal cells, the absence of butyrate produces the same effect. Although an unequivocal *in vivo* demonstration of a direct role for butyrate in the maintenance of normality in colo-rectal mucosal cells has yet to be made, there are supporting data. Thus, raising of large bowel butyrate with a pelletised preparation has been shown to increase apoptosis in aberrant crypt foci in a rat cancer model (Caderni *et al.*, 1998). One form of inflammatory bowel disease, distal ulcerative colitis, may also relate to SCFA metabolism and/or availability. Colonocytes isolated from patients with this condition have an impaired capacity to oxidise butyrate *in vitro* (Burke *et al.*, 1997) and faecal butyrate levels are higher in adult and infant colitis patients than in controls

Table 3. Major Colonic Bacteria, Substrates Utilised and Major SCFA Fermentation Products

Bacteria	Substrates fermented	Major short-chain fatty acids produced		
		Acetate	Propionate	Butyrate
Bifidobacteria	Saccharolytic; Oligosaccharides	✓		
Lactobacilli	Saccharolytic; Oligosaccharides	✓		
Bacteroides	Saccharolytic; some species utilise amino acids	✓	✓	
Eubacterium	Saccharolytic; some species utilise amino acids	✓		✓
Peptostreptococci	Saccharolytic; some species utilise amino acids	✓		✓
Clostridia	Saccharolytic; amino acids; Oligosaccharides*	✓	✓	✓

**C. butyricum* capable of utilising oligosaccharides.

From Mitsuoka 1996; Cummings and Macfarlane 1991; Hill 1995.

(Holtug *et al.*, 1988 ; Treem *et al.*, 1994). This suggests that there is a defect in butyrate metabolism which could be overcome through provision of more substrate because SCFA enemas (including butyrate) have been reported to induce remission in affected individuals but final confirmation is yet to be obtained (Scheppach *et al.*, 1997). Cancer of the large bowel accounts for a large proportion of cancer-related deaths in westernised countries and the incidence is increasing in developing regions, especially in Asia. With increasing affluence, there is a shift in location with large bowel cancers no longer being confined to the most distal region of the colon (Szilagyi 1997), the region where SCFA availability is lowest (Topping and Bird, 1999). Now cancers are being found in greater numbers towards the right (proximal) side of the colon.

The role of SCFA in large bowel physiology raises an important issue which is that both the fermentative substrates and the bacterial population which metabolise them may be secondary in importance to the effective delivery of SCFA, especially to those sites at greatest risk of disease. This is important for RS as it affects the large bowel principally through fermentation and those individuals at greater risk for bowel cancer (designated by the presence of large bowel polyps) were found to be more efficient at digesting and absorbing dietary starch than low risk patients (Thornton *et al.*, 1987).

RS and SCFA Production

Several colonic bacterial groups have the metabolic capacity to ferment starch *in vitro* (Table 3). However, this does not mean that they do so *in vivo* as all of the influences (gelatinisation, amylose content, retrogradation) which contribute to RS in the small intestine apply to large bowel amylolysis. That RS modified large bowel bacterial activity was shown by early studies in rats where high amylose starches (Morand *et al.*, 1992) or legumes (Key and Mathers, 1995) raised total caecal digesta mass and SCFA concentrations and pools. *In vitro* studies showed that starch fermentation by faecal inocula appeared to favour butyrate production (Weaver *et al.*, 1992). Human feeding trials accord with these findings. Increasing the effective intake of RS either through inhibition of small intestinal starch digestion with acarbose (Scheppach *et al.*, 1988) or feeding foods high in RS (Noakes *et al.*, 1996; van Munster *et al.*, 1994) raises faecal butyrate.

These data have attracted much interest in view of the potential role of butyrate in promoting a normal colonocyte phenotype. They may help to account for some of the epidemiological data linking diet to lowered risk of

large bowel cancer. Much of the early epidemiological work in Africa linked their consumption of unrefined plant foods to diminished risk of this malignancy and other diseases (Burkitt, 1995). Accordingly, considerable research effort was directed at the protective effects of fibre itself and there have been a large number of human population studies to determine its role. A *meta*-analysis of these studies showed, rather surprisingly, that the reduction in risk was relatively modest (Trock *et al.*, 1990). A subsequent analysis of published studies showed that substantial protection was conferred by increased starch consumption (Cassidy *et al.*, 1994). A recent study has shown that a low-risk population of Africans had lower fibre but higher starch intakes than a high-risk population of Europeans (O'Keefe *et al.*, 1999). The Africans had higher rates of large bowel fermentation as measured by breath H₂ evolution which implies greater SCFA production. However, it appears simplistic to suppose that increased RS consumption will automatically confer benefit. There are not only a large number of determinants of starch digestibility but also individual variations in the microflora. *In vitro* data show that faecal inocula from some subjects are less capable of fermenting starch than others (Weaver *et al.*, 1989). This has been confirmed in a more recent study (Cummings *et al.*, 1996) which suggested that the variability was a property of both the individual's microflora and the type of RS. It remains to be established which properties of the starch and the mix of bacterial species in the large bowel ecosystem allow amylolysis to proceed. Other animal studies have shown that the absolute levels of SCFA, the relative proportions of the major acids and their distribution along the colon varied between different sources of RS (Anison and Topping, 1994). Overall, these observations have led to the hypothesis that specific types of starch may be especially effective in raising butyrate (Topping and Bird, 1999). One of these appears to be maize as the *in vitro* studies, in which preferential production of this acid was shown, used starch from this cereal.

Starches, RS and the Microflora

Starches can influence the gut microflora simply through the provision of extra substrate. This may occur in the mouth as fermentable carbohydrates (particularly sucrose and other disaccharides and monosaccharides) have been implicated in caries formation (Bibby, 1982). The highly gelatinised starches found in processed foods may be even more important. Highly gelatinised starches stick to teeth on chewing (Åkerberg *et al.*, 1998) and oral carboxylic acid production is greater with foods which favour this adhesion

(Kashket *et al.*, 1996). It seems that factors affecting starch adhesion and/or fermentation could lower caries risk so that processed and unprocessed foods containing RS would affect risk of cariogenesis.

Adverse reactions to RS have been reported under some circumstances. One area of considerable economic significance is the weaning of piglets where significant mortality accrues from swine dysentery due to a specific organism, *Serpulina hyodysenteriae*. Feeding of RS and fermentable NSP (guar gum) leads to raised large bowel SCFA, colonisation with this bacterium and appearance of pathological clinical symptoms (Pluske *et al.*, 1998). These diets increase the number of total culturable bacteria and colonisation by synergistic *Fusobacterium* (Durmic *et al.*, 1998). It should be noted that commercial weaning of pigs is effected very abruptly through introduction of the new diet. It may be this rapid transition, and the concomitant destabilisation of the enteric microflora, which contributes to the syndrome. Possibly, the phenomenon may be relevant to human gut disease as starches (and NSP) have been implicated also in human IBD. Total carbohydrate (starch and sugar) consumption seems to be higher in IBD patients than in controls (Tragnone *et al.*, 1995). An interaction between the gut microflora and the mucosa is a possible contributor to this condition which could be enhanced by fermentable carbohydrate. Immunoreactive antibodies to specific organisms, specifically *Klebsiella*, have been found in patients with ankylosing spondylitis, a population affected also by IBD (Tiwana *et al.*, 1997). A diet low in starch has been shown to lower circulating IgA in these patients leading to the suggestion that the presence of potentially pathogenic organisms in the faecal microflora may be a trigger for these conditions (Ebringer and Wilson, 1996). If this were to occur, it could be a serious contraindication for greater RS consumption. However, further study has not supported a primary role for *K. pneumoniae* in spondylitis (Smith *et al.*, 1997) or this organism, *Escherichia coli* or *Listeria monocytogenes* in IBD (Walmsley *et al.*, 1998). Indeed, it appears that these responses may be a consequence of active disease rather than causative. Removal of RS from the diet induces temporary remission through bowel rest as does a low fibre diet (Klurfeld, 1999). Sulphur-reducing bacteria have been implicated in UC through the production of H₂S and hence blocking of SCFA metabolism by colonocytes (Roediger *et al.*, 1997). This remains to be established as does any role for RS. Disordered fermentation and, possibly, a disordered large bowel microflora have been implicated in IBS. It has been reported that both faecal SCFA concentrations and production rates *in vitro* are lower in patients with diarrhoea-predominant IBS (Treem *et al.*, 1996). More recently, a study *in vivo* showed that H₂ evolution was more rapid in IBS patients although total gas production was the same as in affected individuals (King *et al.*, 1998). Both of these interesting studies were performed with very limited numbers of volunteers and require replication with larger populations.

Modulation of the Gut Microflora and the Role of Starches

The majority of colonic bacteria are saccharolytic, and several bacterial groups, including *Eubacterium*, *Bacteroides*, *Bifidobacterium* and *Escherichia* can

ferment starch (Brown *et al.*, 1998; Wang and Gibson 1993). *Clostridium butyricum* and species of *Bifidobacterium* in particular are effective starch utilisers *in vitro* (Brown *et al.*, 1998). There are data from studies with "human microflora-associated rats" showing that a retrograded high amylose starch raised large bowel butyrate and the numbers of lactobacilli and bifidobacteria (Silvi *et al.*, 1999). These data demonstrate the potential for RS to modify the microflora specifically (*i.e.* to act as prebiotics).

Probiotics and the Role of Starches

Probiotic use centres on a specific colonisation of the human (or animal) gastrointestinal tract by one or several exogenous bacterial species which act to improve health (Naidu *et al.*, 1999). Probiotic organisms commonly used in commercial foods are strains of the lactic acid bacteria *Lactobacillus* and *Bifidobacterium*. *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus* are used in yoghurt manufacture. Demonstrated physiological effects resulting from regular ingestion of probiotic bacteria include immune system stimulation (De Simone 1993), modulation of the enteric microflora (Fooks *et al.*, 1999) and decreased faecal bacterial enzyme activities (Rafter, 1995). Probiotics have been claimed to be effective for preventing or treating a raft of intestinal and systemic disorders including infantile and travellers' diarrhoea, lactose maldigestion, hypercholesterolemia, constipation, and colon and other cancers (Goldin 1998; Sanders 1993). However it must be recognised that much of the investigation has been in animals and there have been relatively few well-controlled trials in humans. In the absence of such studies, many of the effects of probiotics remain unsubstantiated. When one effect, reduction of plasma cholesterol, was examined in humans no effect of probiotics was seen despite positive results in rodents (de Roos *et al.*, 1999). Possible therapeutic use of probiotic lactic acid bacteria (LAB) to control *H. pylori* appears feasible. Various LAB strains have been shown to inhibit the growth of *H. pylori* *in vitro* (Aiba *et al.*, 1998; Michetti 1999) and *Lactobacillus johnsonii*, administered to humans for 14 days in a randomized, double-blind clinical trial, suppressed but did not eradicate *H. pylori* infection (Michetti *et al.*, 1999). The latter is an important finding given the potentially adverse outcomes of complete eradication, as referred to earlier. The inhibitory effect of probiotics on *H. pylori* may be due to the bactericidal actions of organic acids and other substances released by the probiotic microorganism (Brassart and Schiffrin 1997) or from direct competition of the probiotic with *H. pylori* for adhesion sites on gastric mucosal cells (Kabir *et al.*, 1997). Effective use of probiotics in humans is limited by the apparent refractoriness of the human gut microflora to exogenous bacteria resulting in only relatively transient colonisation. In part this could reflect substantial loss of viability of the organisms on passage through the relatively hostile environment of the stomach and small intestine. This drawback has led to the development of the concept of prebiotics.

Starches as Prebiotics

Prebiotics are defined as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and (or) activity of one or a limited number of bacterial species already resident in the colon, and, thus,

Table 4. Counts of Faecal Bifidobacteria in Pigs Fed Diets Containing Low or High-Amylose Starches With and Without Fructooligosaccharides

Amylose Level	-FOS	+FOS
	Bifidobacteria log cfu/g faeces	
Low	10.35	11.00
High	11.74	12.02

Significance level of effects of starch ($P < 0.01$) and FOS ($P < 0.01$).
From Brown *et al.*, 1998.

attempt to improve host health" (Gibson and Roberfroid 1995). Perhaps one of the best known of these agents is fructo-oligosaccharide (FOS) but certain other carbohydrates appear to have prebiotic potential. Starch and NSP have been regarded as unlikely prebiotics, largely because many of the colonic bacterial species are capable of metabolising these carbohydrates. However, several *in vivo* studies in experimental animals and humans suggest otherwise. Alginates, which are used widely in the food industry, appear to be prebiotics (Terada *et al.*, 1996) and RS may also be useful. During studies with a high amylose RS it was noted that the granules developed a particular etching pattern on passage through the upper gut of either pigs or humans (Topping *et al.*, 1997a). This resulted in surface pitting (Figure 1a, b) and it was thought that this could enhance the viability of probiotics by providing a surface for physical adhesion. It was confirmed that specific bifidobacteria adhered to this starch (Figure 1c) (Brown *et al.*, 1998). Feeding trials in mice (Brown *et al.*, 1998) and pigs (Brown *et al.*, 1997) showed that faecal excretion of bifidobacteria was higher when the animals were fed live *Bifidobacterium longum* with this high amylose starch. In pigs, faecal bifidobacterial numbers rose from 10.76 CFU/day in pigs fed a control (waxy maize) starch to 11.73 CFU/day in animals fed the high amylose starch (Brown *et al.*, 1997). Total faecal SCFA and butyrate excretion was significantly higher when the pigs were fed the high amylose starch. In the absence of *B. longum*, excretion of total SCFA and butyrate were 25.4 and 3.2 mmol/day respectively with low amylose starch, and 60.7 and 9.1 with the high amylose starch. There was no effect of the probiotic on SCFA levels. A further experiment showed that the effects of FOS and the RS on faecal bifidobacteria numbers were additive (Topping *et al.*, 1997b) - suggesting that they acted by different mechanisms (Table 4). Possibly the former acted as a substrate and the latter as a physical protector. This concept is supported by the longer survival and maintenance of high numbers of viable organisms in yoghurts containing high amylose starch where the effective shelf life was improved by several weeks (Brown *et al.*, 1998). Prebiotic action does not seem to be limited to high amylose starches *per se* but also to chemically modified variants (*i.e.* RS4) which are also effective. Studies *in vitro* have shown that bifidobacteria can adhere to starches modified through acylation, octenylsuccinylation, carboxymethylation and succinylation (Brown *et al.*, 1998). This adhesion varied by strain and did not extend to *Lactobacillus casei*. These data show that RS2 and RS4 have the potential to act as prebiotics – an attribute which could be especially useful in delivering organisms to regions where they are needed (*e.g.* to the distal colon). Several

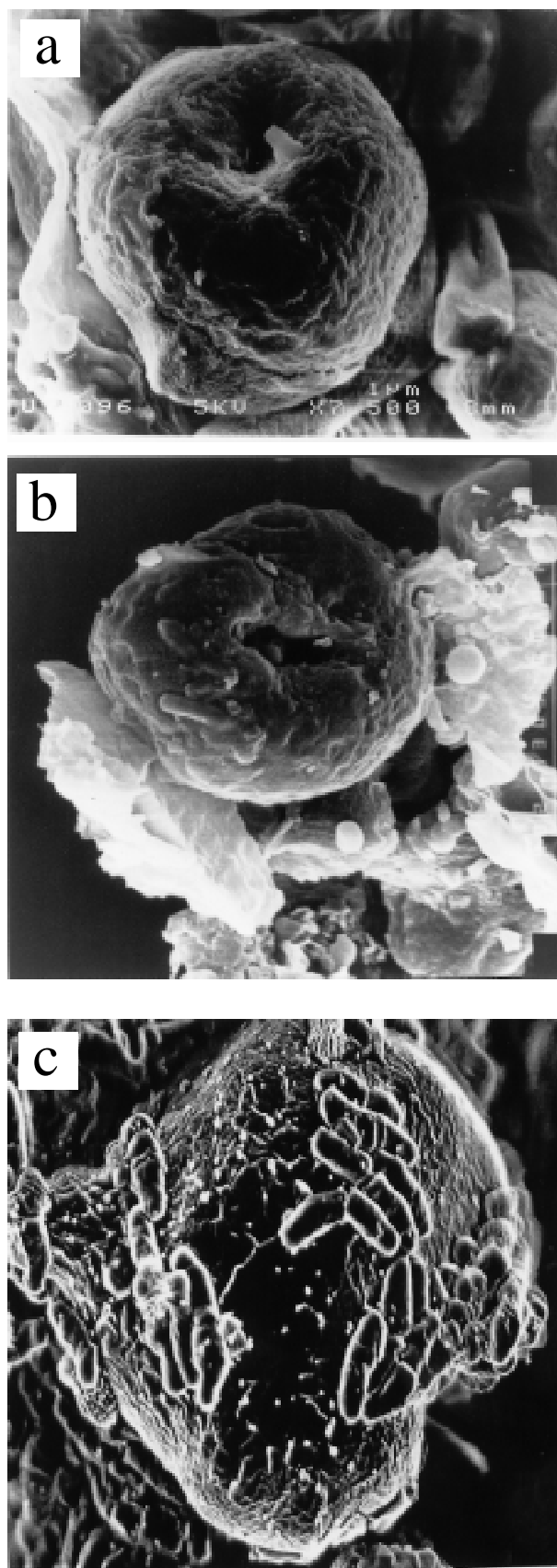


Figure 1. High amylose starch granule after passage through (a) human and (b) porcine small intestine and (c) showing adherent bifidobacteria.

other studies support the prebiotic action of RS and that the effect varies with type. Kleessen *et al.*, (1997b) have shown that retrograded potato starch (RS3), but not native potato starch (RS2), promotes faecal and caecal *Lactobacillus* counts in rats. A preliminary report showed that consumption of foods containing high amylose starch (RS2) by volunteers resulted in lower faecal counts of *Bifidobacterium*, and higher butyrate levels, compared to a cornflake control diet (Rao *et al.*, 1997). In young pigs fed rice-based diets formulated to increase the quantity of starch (RS1 and 3) flowing into the caecum, counts of bifidobacteria and lactobacilli in the proximal colon were similar to those in pigs fed a digestible starch diet (Bird *et al.*, 1997). Numbers of coliforms, especially *E. coli*, were several log units lower in pigs offered the RS-rich diet. These limited data suggest that certain starches alter the species composition and metabolic activity of the colonic microbiota. Not only is the total amount of starch reaching the colon important, but the chemical and physical properties of the resistant starch seem to be important in determining the prebiotic properties of RS.

Infant SCFA and Weaning to Starches

Probiotics do not seem to modify faecal SCFA (*e.g.* Brown *et al.*, 1997) and, in any event, on current evidence they would seem unlikely to do so. Bifidobacteria and, to a lesser extent, lactobacilli are found in the human gastrointestinal system in their relatively greatest numbers in milk-fed infants (Mitsuoka, 1996). Studies using NMR and ¹³C-glucose as a substrate have confirmed that in infants a bifidobacterial fermentation appears to predominate (Wolin *et al.*, 1998a). Examination of faecal samples from these infants show also that the SCFA profile is unlike that in adults (Edwards *et al.*, 1994). Although acetate is the predominant SCFA in adults and pre-weaned bottle or breast-fed infants, propionate is present at much lower concentrations in the latter while butyrate is virtually absent. Other products such as ethanol, formate, succinate and lactate appear in infant faeces but not in appreciable quantities in adults (Wolin *et al.*, 1998b). These metabolites may play an important role in controlling infection while the absence of butyrate could be significant for neonatal gut development. Faecal butyrate is rather higher in bottle-fed babies than in those receiving breast milk and the former may be at greater risk of developing IBD in later life than infants who were breast-fed (Szilagy, 1998). However, this predisposition may stem from a heightened sensitivity to milk formula proteins in infancy rather than any effect of butyrate. In adults, SCFA are trophic and appear to play a role in promoting a normal colonocyte phenotype. Diets high in a high amylose starch promote colon length in adult pigs (Topping *et al.*, 1997a) and the increase in length is proportional to the amount of resistant starch entering the colon (Bird AR, Trimble RP, Brown, IL, Topping DL and Illman RJ - unpublished observations). It appears reasonable to assume that this reflects greater SCFA availability.

In infants, the role SCFA play in adults in promoting intestinal growth appears to be accomplished instead by growth factors in milk and those expressed within the colon. Insulin-like growth factors (IGF-I and IGF-II) are important regulators of somatic cell growth and differentiation and gut tissues seem to be particularly responsive to their

trophic actions. IGF-I promotes tissue accretion in the small and large bowel of normal suckling (Steeb *et al.*, 1997) and young adult rats (Conlon *et al.*, 1995) and IGF-II appears to have similar although less potent effects than IGF-I (Conlon *et al.*, 1995). However, not all studies have confirmed an increase in gut size in response to IGF-I administration (Bird *et al.*, 1994a; Young *et al.*, 1990). Short-term subcutaneous administration of long R³ IGF-I, a potent IGF-I analog, decreased overall length of the small intestine in mice and tended to reduce the thickness of the bowel wall (Bird *et al.*, 1994a). In mice, short-term systemic administration of another growth factor, epidermal growth factor (EGF) had no effect on small bowel mass and length, or on jejunal architecture, yet this peptide elicited a substantial increase in active jejunal glucose absorption (Bird *et al.*, 1994b). In humans, foetal plasma IGF concentrations increase with gestational age, especially during the final trimester although levels are markedly less than those found in adults (Lee and Han 1990). Concentrations continue to rise during infancy and childhood. Plasma IGF-I levels reach a maximum at puberty, and then decline progressively with age (Cohen *et al.*, 1992, Sara and Hall 1990, Yamamoto *et al.*, 1991). Plasma IGF-II concentrations are relatively low before birth and increase during the first year of life (Lee and Han 1990, Sara and Hall 1990). In adults, IGF-II is present in the bloodstream at levels fourfold greater than those of IGF-I (Sara and Hall 1990). It appears reasonable to assume that the weaning process leads to a shift in the large bowel towards SCFA both as metabolic substrates and as modulators of development and physiological activity. Maintenance of a pre-weaning colonic population of bacteria (and their fermentation products) in adults may not be desirable for long term bowel health. Similarly, maintenance of elevated plasma IGF-I may predispose to adenocarcinoma of the colon (Holly *et al.*, 1999). Lower plasma levels of IGF binding protein (IGFBP), especially IGFBP-3, can increase the tissue availability of IGF-I (Ma *et al.*, 1999). However, SCFA seem to interact with them to lower the risk of disease. Thus, in well differentiated cells of the colonic cancer cell line CaCo-2, butyrate stimulates the secretion of IGFBP-2 without altering that of IGFBP-3 (Nishimura *et al.*, 1998). Butyrate also appears to lower the *in vitro* responsiveness of colon cancer cells to EGF (Archer *et al.*, 1998). This could contribute to diminished tissue availability of IGF and so may modulate the carcinogenic process. Studies in animals have shown that IGF I and II are highest in neonates and decline with age. When adult rats were fed dietary fibre as wheat bran, caecal epidermal growth factor (EGF) levels were lower than in animals fed a low-fibre diet (Schaudies *et al.*, 1991). This change was accompanied by an increase in the mass of cecal tissue. Under these circumstances wheat bran raises digesta concentrations of SCFA, including butyrate (Topping *et al.*, 1993) which would be expected to increase substrate supply and also promote colonocyte growth and turnover. It could be hypothesised that fermentation provides SCFA which obviates the need for growth factors and leads to down regulation of their synthesis. If this is correct, it has a number of important corollaries. The first is to add support for breastfeeding as faecal butyrate concentrations in infants fed by this route are lower than in bottle-fed infants. Secondly, the weaning process itself could be optimised by the consumption of foods which

promote a favourable bacterial profile while enhancing the production of SCFA, especially butyrate. RS (and other prebiotics) could assist in risk reduction by enhancing probiotic delivery and the generation of a favourable SCFA profile in the colon.

Recently, there has been concern about RS4 in infant foods (Lanciers *et al.*, 1997) even though they may be prebiotics. As acylated SCFA, they may deliver SCFA to the large bowel (Annison *et al.*, 1998). Thus, their presence in infant foods may be of assistance in the maturation of the developing large intestine. The final corollary is that efforts to modify the large bowel microflora so as to raise the populations of lactobacilli and bifidobacteria would be counterproductive if they were to lower the production of desirable products such as SCFA.

Conclusions and Future Directions

Starches and RS are emerging as important factors in health, especially through their interactions with gut bacteria. These interactions seem to occur both physically and through the metabolism of starches by the microorganisms. It appears likely that starch fermentation in both the mouth and the large bowel can be modulated so as to lower disease risk. In the mouth, slower starch breakdown can reduce caries risk. In the colon, fermentation can promote SCFA production so as to optimise physiological function and manage and prevent important pathologies. It appears likely that the latter will involve tailoring the supply of RS to meet individual variations in the microflora which may limit starch fermentation. Newer varieties of starch high in RS are one of the means whereby this can be achieved. In this regard the parallels with NSP are striking. Dietary fibre used to be regarded as a single entity with multiple actions. Now, different NSP are known to affect gut physiology in disparate ways. The same appears to be true for RS1-4. Thus, identifying the factors which mean that the mix of bacteria in the microbial population which metabolise the various forms of RS appears to be an important research objective. Equally, identifying the ways in which the various types of RS can assist in delivering probiotics to the upper gut (to deal with infections) and to the large bowel (to optimise the microflora and to deliver SCFA) also is important. The relationships between the microflora in preweaned infants and those in adults, especially as influenced by starches seems to be important in promotion of gut health throughout life. The gaps in knowledge in these significant aspects of gut physiology are large and warrant exploration, especially as they relate to the gut microflora. Much of the technology (animal models, human faecal and *in vitro* measures, chemical and biological assays) is available to study microbial starch metabolism but the same cannot be said about the impact of starch on the microflora. While conventional culture and microscopic methods have provided the basic knowledge of the large bowel microflora, they are limited both in scope and their demands for labour (Tannock, 1999). It is to be hoped that the application of new molecular methodologies will give a fuller understanding of the ways in which starches and other carbohydrates influence bacterial populations in the human gut.

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