NUTRITIONAL INFLUENCES ON INTERACTIONS BETWEEN BACTERIA AND THE SMALL INTESTINAL MUCOSA

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INTRODUCTION

Much recent research in intestinal bacteriology has been based on the tenet that the indigenous microflora plays an important role in preserving the health of individuals by preventing enteric colonization by opportunistic pathogens. This colonization resistance

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may be a consequence of competitive exclusion of pathogens by the indigenous flora. In other situations the control exerted by the indigenous flora is more subtle and may involve the synthesis of molecules which non-specifically stimulate the immune system or chemically interfere with the interaction between pathogens and intestinal epithelia. The concept is evolving that natural or synthetic antiadhesive compounds (soluble receptor analogues) might provide an alternative approach to rational drug design in antibacterial therapy and this has fuelled a widespread research interest in molecular interactions at the intestinal–bacterial interface. The role of nutrition in augmenting or inhibiting these interactions represents the major discussion point of this review.

COLONIZATION OF THE INTESTINAL TRACT

The intestinal tract and other mucous membranes are continually contaminated by micro-organisms from the environment. Through a combination of synergic and antagonistic interactions (Linton & Hinton, 1990) steady state is attained and the resultant bacterial population is variously referred to as the natural, indigenous, resident, normal or commensal microflora.

It has been estimated that several hundred microbial species inhabit the mammalian alimentary tract (Finegold *et al.* 1983). To provide a comprehensive description of the resident microbiota in the developing or adult intestine is a formidable task, and it is still only possible to indicate relative frequency and proportions. Study of the enteric flora is further complicated by the fact that within the small intestine there are several habitats including the villus surface, crypts, epithelial associated mucins and luminal mucus. Very different bacterial species colonize these diverse intestinal niches. The normal microbiota in a single community may also differ from that of another of the same species due to differences in diet, husbandry or climatic conditions. In spite of the technical difficulties several authors have attempted to describe, to varying degrees, the bacterial composition of the intestine of mammalian species (for reviews see Moughan *et al.* 1992; Maxwell & Stewart, 1994).

The selection and establishment of a stable intestinal microflora is dependent on several factors including local immune mechanisms, interbacterial interactions, the presence of bacterial receptors on mucosal surfaces, availability of nutrients from endogenous and dietary sources, digesta flow, conditions of pH, oxygen–reduction potentials and the availability of molecular oxygen within the intestine (Stewart *et al.* 1993).

THE GLYCOCONJUGATE CHEMISTRY OF INTESTINAL SURFACES

The differentiation processes of intestinal epithelial cells are associated with important changes in the synthesis and expression of membrane and secretory glycoproteins and glycolipids. This diversity is a feature not only of intestinal cell differentiation on the crypt–villus axis but also of membrane and mucin constituents in proximal and distal regions of the small intestine. The oligosaccharide structures of intestinal glycoconjugates are not themselves primary gene products but are constructed in a stepwise manner, monosaccharides being added to precursor oligosaccharides *via* several glycosyltransferases encoded by different genes (Neutra & Forstner, 1987; Ito & Hirota, 1992). The maturing glycoconjugate complexion of each intestinal cell is influenced by many different factors such as the intrinsic composition of glycosyltransferase species defined by the genotype of

the individual, the relative activity or amount of these enzymes (repression or induction of the enzymes), competition between enzymes with overlapping substrate specificity, the organization of the enzymes in Golgi membranes, utilization of precursors and specific substrate sugars, and the activity level of degrading enzymes (Ito & Hirota, 1992). In spite of this considerable potential for diversity, glycosylation processes within the intestinal epithelium frequently proceed in a predictable fashion.

MEMBRANE GLYCOCONJUGATES

Temporal glycosylation changes have been extensively investigated in both rats and pigs. In these species a progressive change from α2,6 sialylation to α1,2 fucosylation of microvillar glycoconjugates occurs during postnatal development (Taatjes & Roth, 1990; King & Kelly, 1991; King *et al.* 1993). Cytochemical investigations indicate that intestinal membrane oligomannose-type *N*-linked oligosaccharides in the developing pig intestine are replaced by complex or hybrid *N*-linked structures (T. P. King, R. Begbie & D. Kelly, unpublished observations). In humans and many other mammalian species important differences in the glycosylation of intestinal membranes may be correlated with ABO histo-blood groups and are thus genetically mediated (Oriol, 1987; King & Kelly, 1990, 1991).

MUCIN GLYCOCONJUGATES

Intestinal goblet cells synthesize and secrete high molecular weight glycoproteins called mucins. Upon secretion, mucins hydrate and gel, generating a protective mucus blanket overlying the epithelial surface (Specian & Oliver, 1991). Non-mucin constituents of the mucus blanket include water, serum and cellular macromolecules, electrolytes, sloughed off epithelial cells, cell debris and other glycoproteins. A high percentage of the weight of mucin glycoproteins consists of oligosaccharides, *O*-linked to multiple serine or threonine residues in the polypeptide backbone. In addition, a growing number of mucins are reported to contain a small number of *N*-linked glycans (Strous & Dekker, 1992). In the human and pig small intestine immature goblet cells deep within the crypts produce neutral mucins containing little sialic acid (Specian & Oliver, 1991; King, 1994). As they mature and migrate to the villus tip, the mucins become increasingly sialylated; these sialic acid residues not only increase the acidity of the molecule but are also sites for further modification by *N*- and *O*-acylation (Filipe & Fenger, 1979). Age related changes in the glycosylation of goblet cell mucins are also a conspicuous feature of intestinal development in neonates (King & Kelly, 1990; King, 1994).

BACTERIAL ADHESINS AND INTESTINAL RECEPTORS

Many indigenous and pathogenic bacteria specifically adhere to complex carbohydrates of small intestinal membrane and mucin glycoconjugates. The diversity of these carbohydrate receptors plays an important role in host range, tissue tropism and the triggering of host responses (Hultgren *et al.* 1993). This is particularly noticeable in neonates where both beneficial and deleterious alterations in the microbial balance can accompany ontogenic epithelial glycosylation changes (Kelly *et al.* 1992; Stewart *et al.* 1993). The relationship may be passive and involve bacterial colonization mediated through binding to expressed glycoconjugates or may involve chemical modification of inhospitable sites through the

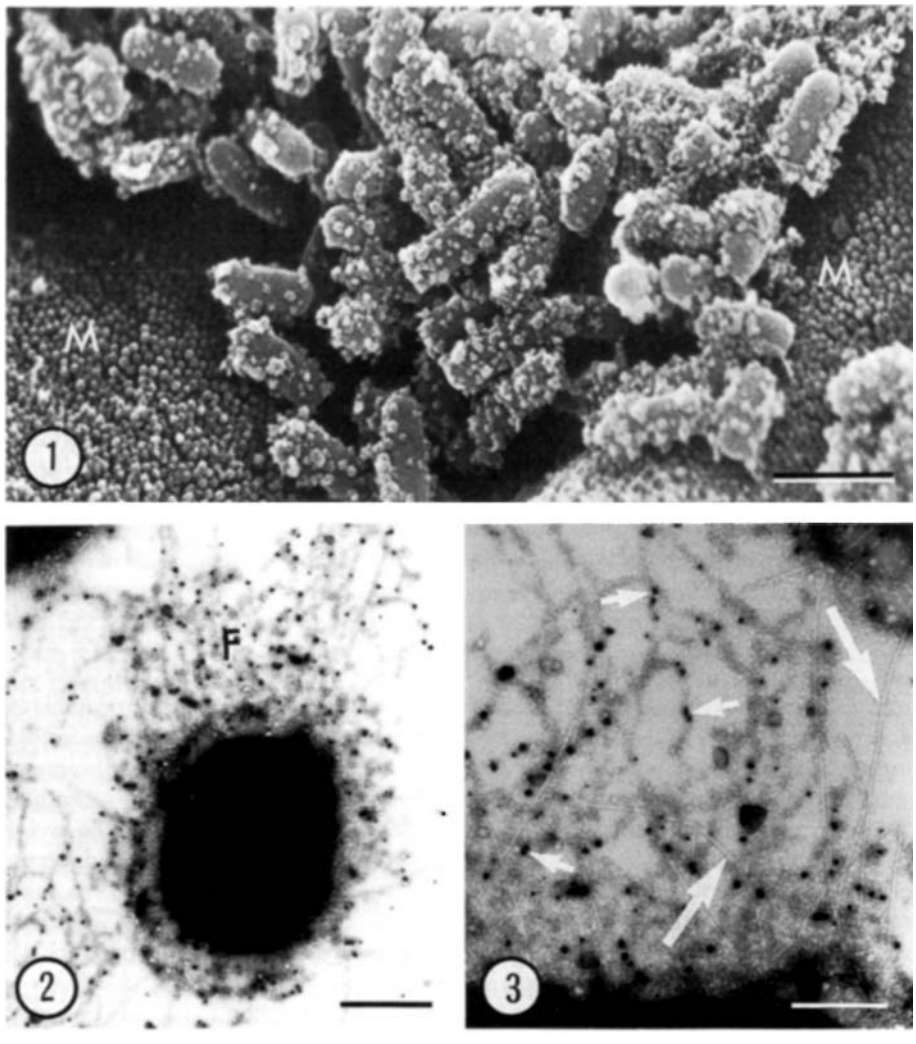


Fig. 1. Scanning electron micrograph showing K88ac ETEC on jejunal enterocyte surface of a newly weaned pig. K88 fimbriae are immunolabelled with an anti-K88a monoclonal antibody followed by immunogold cytochemistry and silver enhancement to produce a particulate label on the surfaces of the bacteria. M = tips of the intestinal microvilli, bar = 2 μm.

Fig. 2. Transmission electron micrograph of negatively stained whole mount of K88ab ETEC showing surface fimbriae (F). The presence of K88 fimbriae is revealed by immunogold/silver labelling, bar = 500 nm.

Fig. 3. Higher magnification of preparation in Fig. 2 showing type-1 fimbriae (large arrows) and aggregations of K88 fimbriae (small arrows and immunogold/silver labelling), bar = 250 nm.

actions of secreted exoglycosidase enzymes. Important bacterial surface factors involved in mediating adherence to intestinal surfaces include fimbriae (pili), outer membrane proteins and lipoteichoic acids.

FIMBRIAL ADHESINS

The pioneering studies of Duguid and his collaborators (Duguid & Old, 1980) demonstrated that many bacterial species, particularly those of the family Enterobacteriaceae, possess the

ability to agglutinate red cells and that the bacterial agglutinins can be consigned to one of two groups designated mannose sensitive or mannose resistant depending on whether or not the haemagglutinating activity is mannose inhibitable. In many bacteria it has been determined that the mannose sensitive and mannose resistant agglutinins are located on surface fimbriae (Hultgren *et al.* 1993).

Type-1 fimbriae

Mannose sensitive fimbriae, usually termed type-1 fimbriae, are associated with several enteric species. Close serologic relationships exist among type-1 fimbriae of *Escherichia*, *Klebsiella* and *Shigella* spp., whereas fimbriae of *Salmonella* and *Citrobacter* species constitute a second group. In addition, the type-1 fimbriae of *Enterobacter*, *Edwardsiella*, *Hafnia*, *Serratia* and *Providencia* each comprise a distinct serologic group (Clegg & Gerlach, 1987). Type-1 fimbriae have the form of straight, tubular structures up to 2 μm in length and 7–8 nm in diameter. Structurally they are composed of polymers of FimA, a 17 kDa structural protein arranged in a right-handed helical array surrounding a hollow axillary core (Brinton, 1965). Three ancillary proteins, FimF, FimG and FimH, are also assembled as minor components of the filaments. FimH is thought to be primarily responsible for the lectin-like adhesive properties of the fimbriae, but association with FimG appears to be necessary for activity (reviewed by Sokurenko *et al.* 1992).

The adherence of *E. coli* O157:H7 strains to rabbit ileal brush borders is mediated by type-1 fimbriae binding to α-linked mannosyl residues present on surface glycoproteins (Durno *et al.* 1989). Rat intestinal mucin bears oligomannosyl receptors for the same *E. coli* type-1 fimbriae and it has been shown that these receptors are located on *N*-linked oligosaccharides of the 118 kDa link glycopeptide region of the mucin (Sajjan & Forstner, 1990*a*, *b*). *In vitro* investigations by Aslanzadeh & Paulissen (1990) indicated that type-1 fimbriae are involved in the adherence and pathogenicity of *Salmonella enteritidis* in mice. Conversely, Lockman & Curtiss (1992) have suggested that type-1 fimbriae are not virulence factors for colonization of the mouse small intestine by *Salmonella typhimurium*.

The role of type-1 fimbriae in enteric disease has been difficult to ascertain and remains the subject of some controversy (Holland, 1990; Krogfelt *et al.* 1991). These organelles are expressed by a large fraction of clinical isolates of the Enterobacteriaceae and it is perhaps this ubiquity that makes determination of their role in virulence more difficult (Sokurenko *et al.* 1992).

Fimbrial serotypes associated with diarrhoeal disease

Acute infectious enteritis caused by enterotoxigenic *E. coli* (ETEC) is a major cause of morbidity throughout the world. The disease is often mild and self limiting in healthy adults, but in the malnourished, in the aged and in young children and animals the symptoms may be severe (Gross, 1990). The virulence of ETEC is multifaceted and is attributable to both the production of fimbrial adhesins (Figs 1–3) and the elaboration of enterotoxins which bind to specific intestinal membrane receptors. The carbohydrate binding specificity of many ETEC strains responsible for the induction of diarrhoea in animals and man does not depend on the presence of mannose in the receptor structure.

Two important host specific adhesion fimbriae designated colonization factor antigens I and II (CFA/I and CFA/II) have been identified in some human ETEC strains (Evans & Evans, 1978). CFA/I fimbriae consist of only one type of adhesive subunit, of which only one at the tip is accessible to the receptor (Buhler *et al.* 1991). There is evidence that CFA/I fimbriae recognize the sialylated ganglioside GM2 (Faris *et al.* 1980). Three distinct antigens have been identified within the CFA/II group: coli surface antigen 1 (CS1), CS2 and CS3. CS1 and CS2 are 6–7 nm in diam., long rigid fimbriae similar to CFA/I, whereas CS3 fimbriae are thinner, flexible fimbrial structures, 2–3 nm in diam. (Knutton *et al.* 1985).

CFA/IV (PCF8775) expresses three coli surface antigens (CS4, CS5 and CS6) that appear on *E. coli* of specific serogroups and that seem to have individually distinct haem-agglutination characteristics (Holland, 1990). There is little available information concerning the receptor characteristics of CS antigens although the CS3 antigen is believed to recognize oligosaccharide structures found on lactosaminoglycans, either on their backbone sequences or as subdeterminants of antigens related to histo-blood groups (Neeser *et al.* 1989).

K88 fimbrial adhesins associated with diarrhoea in piglets are plasmid encoded and are the major constituents of fine fimbriae of diam. 2–3 nm. K88 ETEC infections characteristically peak one week after birth and in the postweaning period although K88 receptors are present on the intestinal membranes of both neonates and adults (Harel *et al.* 1991; Mouricout, 1991). This suggests that susceptibility to K88 ETEC infection is complex and involves the interaction of luminal factors as well as receptor expression (Conway *et al.* 1990; Willemsen & de Graaf, 1992). K88 ETEC have been shown to adhere both *in vivo* and *in vitro* to enterocytes of K88 susceptible piglets but not to those of any K88 resistant pigs (Sellwood, 1979; Cox & Houvenaghel, 1993). Five porcine phenotypes can be distinguished with regard to brush border adhesiveness with K88ab, K88ac and K88ad serotypes (Bijlsma & Bouw, 1987). Differences in these five variants are likely to be due to the presence of highly specific and accessible brush border receptors (Bijlsma *et al.* 1982; Erickson *et al.* 1992). The precise molecular nature of these receptors has not yet been established. K88ab adhesins adhere to murine brush border membrane proteins with M_r of 57, 67 and 91 kDa (Laux *et al.* 1986). In the pig, glycoproteins of 67 kDa have been implicated in K88ab and ac adhesion while the K88ad receptor has been identified as a 40 kDa glycoprotein (Mouricout, 1991). Porcine brush border glycoproteins ranging in size from 40 to 70 kDa were recently shown to bind K88 fimbriae (Willemsen & de Graaf, 1992). Erickson *et al.* (1992) identified two porcine brush border glycoproteins (210 and 240 kDa) that bind K88ac fimbriae. The authors suggested that the presence of these glycoproteins on adhesive brush borders and absence on non-adhesive brush borders may be the basis for resistance and susceptibility of pigs to K88ac *E. coli* infections. Lower molecular weight proteins (37 and 48 kDa) were shown to bind biotinylated and ^{35}S-labelled K88ac adhesin but this binding was not specifically blocked by the presence of molar excess of unlabelled K88ac adhesin and the proteins were detected in both non-adhesive and adhesive phenotypes. Although the 37 and 48 kDa proteins do not fulfil the criteria for phenotypically important receptors, these proteins may be low affinity receptors that promote the initial interaction of K88ac *E. coli* with the phenotypically important 210 and 240 kDa brush border receptors (Erickson *et al.* 1992). Gibbons *et al.* (1975) concluded that an unsubstituted β-galactosyl residue was an important feature of the structural chemical requirements for binding the K88 adhesin. Competitive inhibition studies demonstrated that stachyose (Gal α1,6 Gal α 1,6 Glc α1, β2 Fru) and galactan (a polymer of D- and L-Gal) reduced binding of K88 to porcine brush border membranes by 33 and 50% respectively (Sellwood, 1984). Nilsson & Svensson (1983) found that K88 reacted with oligosaccharides containing galactosyl residues isolated from glycolipid fractions of the pig small intestine. Payne *et al.* (1993) recently suggested that β-linked galactose residues may form the molecular basis of both glycoprotein and glycolipid receptors for the K88 fimbrial adhesin in the porcine small intestine.

K99 ETEC strains which cause diarrhoea in newborn calves, lambs and pigs also express fine fimbriae of diam. 2–3 nm (Morris *et al.* 1980). Clinical studies indicate that with increasing host age the small intestine may develop resistance to adhesion mediated by K99 (Runnels *et al.* 1980). K99 *E. coli* express fimbrial adhesins which bind to sialylated glycoproteins and glycolipids (Mouricout, 1991). Two pig phenotypes have been identified,

those expressing high levels of K99 receptors and those exhibiting low levels. Seignole *et al.* (1991) have determined that piglets susceptible to K99 adhesion express higher levels of sialylated glycolipids than those resistant to K99 attachment. Furthermore, the piglet intestinal mucosa contains a substantially higher content of acidic glycolipids than that of adult pigs (Teneberg *et al.* 1990). *N*-glycolylneuraminyl-lactosylceramide (NeuGc-GM3) has been identified in cattle as a major receptor for K99 (Lindahl *et al.* 1987; Teneberg *et al.* 1993). The membrane content of this ganglioside is maximal in newborn pigs and gradually decreases during development (Yuyama *et al.* 1993).

F41 is a chromosomally and/or plasmid encoded ETEC fimbrial adhesin which is often produced by strains which also elaborate K99 adhesins (Harel *et al.* 1991). There is clinical evidence to suggest that the polyfimbriated K99/F41 strains adhere in higher numbers to porcine intestinal villi than strains which only produce F41 or K99 fimbriae (Cox & Houvenhagel, 1993). F41 has a high affinity for terminal GalNac moieties (Lindahl & Wadstrom, 1986; Brooks *et al.* 1989). Cytochemical analysis of the membrane and mucin glycoconjugates in piglets reveals that these sugar moieties occur on immature glycoconjugates in newborn animals but are also present on histo-blood group A antigens in some weaned animals (King & Kelly, 1991).

ETEC strains carrying 987P fimbriae colonize the small intestine and cause diarrhoea in neonatal (> 6 days post partum) pigs but not in older (> 3 weeks) postweaning pigs in spite of the fact that intestinal receptors are present in both groups (Dean *et al.* 1989; Harel *et al.* 1991). A < 17 kDa 987P binding component was present in the intestinal mucus of the older animals but not in neonates and Dean (1990) concluded that this receptor in the mucus may prevent *in vivo* adhesion of $987P^+$ *E. coli* by competing with brush border 987P receptors.

Nagy *et al.* (1992) investigated the adhesion of ETEC strains which did not produce K88, K99, F41 or 987P fimbriae. Colonization of these so-called $4P^-$ strains was characterized by adhesion to porcine intestinal villi overlying Peyer's patches, mediated by fimbriae. Small intestinal adhesion by these isolates was found to be dependent on receptors that develop progressively with age during the first 3 weeks after birth. The carbohydrate specificities of $4P^-$ fimbriae are unknown.

The specific interaction of ETEC strains with the Peyer's patch epithelium may have particular relevance with regard to the delivery of bacterial antigens to the underlying gut associated lymphoid tissue. A wide range of pathogenic Gram-negative bacteria, including *E. coli* RDEC-1, *Vibrio cholerae*, *Salmonella typhimurium*, *Yersinia enterolytica* and *Campylobacter jejuni*, have been shown to adhere selectively or preferentially to M cells of experimental animals. A detailed description of the consequences of transepithelial transport of bacteria and bacterial antigens is beyond the scope of this review but has been recently extensively covered by Kraehenbuhl & Neutra (1992).

Salmonella typhimurium can cause severe diarrhoeal disease in pigs. Frequent reinoculation of the intestines by the faecal–oral route is considered an important mechanism for the persistent colonization of the porcine intestine, although evidence has also been presented that colonization is much enhanced in *Salmonella* strains which possess adhesive fimbriae (Isaacson & Kinsel, 1992). Thin aggregative fimbriae have been localized on the surfaces of several *Salmonella* species (Thorns *et al.* 1990; Collinson *et al.* 1993). The receptor specificities of the fimbrial adhesins have not been determined.

Mucosal adhesion by enteropathogenic *E. coli* (EPEC) involves two distinct stages: (1) initial attachment of bacteria promoted by plasmid encoded fimbrial adhesins and (2) effacement of microvilli, intimate EPEC attachment and cytoskeletal disruption (Knutton *et al.* 1987; Donnenberg & Kaper, 1992). *Vibrio cholerae* colonizes villus surfaces by adhering to peripheral components of the enterocyte glycocalyx but does not come

into close contact with enterocyte microvillar membranes. However, on Peyer's patch M cells *Vibrio* forms tight membrane attachment sites that result in rapid phagocytosis and transport (Winner *et al.* 1991). Commensal Gram-positive segmented bacteria in the rodent ileum colonize mucosal surfaces by forming stable attachment sites on absorptive cells (Kraehenbuhl & Neutra, 1992).

Fimbrial adhesins associated with Helicobacter pylori

Helicobacter pylori is a microaerophilic bacterium found in the stomach and proximal small intestine of asymptomatic humans as well as patients with acid peptic disease and gastric adenocarcinoma (Falk *et al.* 1993; Ofek & Doyle, 1994). Electron microscopic investigations have shown that *H. pylori* can adhere to apical membranes of epithelial cells in a manner reminiscent of the adherence pedestals of enteropathogenic *E. coli* (Ofek & Doyle, 1994). Surface fimbriae mediate the attachment of the bacteria to both gastric membranes and mucin secretions. *In vitro* adherence assays have shown the presence of *H. pylori* receptors on the surface mucous cells in the stomach and on the villus epithelium of the duodenum (Falk *et al.* 1993). *H. pylori* appears to be capable of expressing a number of adhesins with distinct specificities. Some *H. pylori* strains have been shown to cause haemagglutination of erythrocytes from only one species, whereas other strains cause haemagglutination of erythrocytes from several species including humans (Ofek & Doyle, 1994). It has been reported that different *H. pylori* strains recognize a heterogeneous class of sialoglycoconjugates (Lelwala-Guruge *et al.* 1992, 1993; Evans *et al.* 1993). There is an emerging body of data showing that *H. pylori* also bind to non-sialylated receptors. For example, using TLC immunostaining techniques, Saitoh *et al.* (1991) identified both sialylated and non-sialylated glycosphingolipid receptors in the human stomach. The relationship between histo-blood group antigen expression and colonization by *H. pylori* was investigated by Borén *et al.* (1993), who concluded that the Lewis (b) (Le(b))histo-blood group antigen mediates bacterial attachment of the organism to gastric mucosa. Bacteria did not bind to the Le(b) antigen substituted with terminal GalNAcα1,3 residue (histo-blood group A determinant), suggesting that *H. pylori* receptors might be reduced in individuals of blood group A phenotypes, as compared with blood group O individuals. *In vitro* adherence assays, employing blood group antigen specific monoclonal antibodies to block the attachment of *H. pylori* to histological sections of human stomach, showed that the attachment of *H. pylori* to fucosylated histo-blood group positive mucous cells was not dependent upon terminal non-substituted sialic acid residues (Falk *et al.* 1993).

OUTER MEMBRANE PROTEINS

Yersinia enterocolitica is an enteroinvasive bacterium that causes gastroenteritis. A 42 to 50 MDa virulence plasmid of *Y. enterocolitica* encodes a variety of proteins whose expression is regulated by both temperature and the availability of calcium (Mantle & Rombough, 1993). Several of these proteins are secreted, some of which associate with the outer membrane of the organism and are referred to (Michiels *et al.* 1990) as Yops (*Yersinia* outer membrane proteins). In addition the plasmid encodes for YadA, a fimbrial adhesin that is a true outer membrane protein (Kapperud *et al.* 1987). When expressed these plasmid encoded proteins alter the surface charge and hydrophobicity of the bacterium, promote auto-agglutination and mannose resistant haemagglutination and enhance adherence to epithelial membranes (Mantle & Rombough, 1993; Mantle & Husar, 1993). *Campylobacter jejuni* and *C. coli* are major causes of human enteritis. An outer membrane protein of approximately 27 kDa is believed to play a role in adherence of *C. jejuni* to intestinal cells (Kervella *et al.* 1993).

Lactobacilli represent a prevalent group of the indigenous flora of the intestine. For almost a century organisms of this genus have been alleged to augment the protective barrier of the intestine but their mode of action remains speculative. Pedersen & Tannock (1989) demonstrated that adherence of lactobacilli to epithelial surfaces of the digestive tract was a prerequisite for successful colonization and may also represent a key feature of their probiotic action. In a recent model Coconnier *et al.* (1992) proposed that an extracellular proteinaceous component, produced by adhering lactobacilli, provides a divalent bridge that links the bacteria to enterocyte surfaces. The extracellular bridging protein interacts with carbohydrate components of the bacterial cell and the intestinal epithelium. These proteins may also be derived from the host epithelium or introduced as lectin constituents in the diet (Pusztai *et al.* 1990; Tannock, 1992). Although the precise nature of the lectin receptors remains to be elucidated, there is evidence that specificity of different lactobacillus strains may reflect species and age related variations in the glycoconjugate complexion of intestinal surfaces (Tannock, 1992).

LIPOTEICHOIC ACID

The surfaces of Gram-positive bacteria are typically composed of two structurally related, negatively charged components. One is covalently linked to the peptidoglycan and is usually referred to as the teichoic acid or the secondary (or accessory) cell wall polymer (Poxton & Arbuthnott, 1990). The other is anchored in the cytoplasmic membrane and protrudes through the wall. This is the membrane or lipoteichoic acid (LTA) (Poxton & Arbuthnott, 1990). The chemical composition and possible modes of action of LTA were comprehensively reviewed by Christensen *et al.* (1985). LTA is a linear polyglycerophosphate containing a glycerophosphoryl–diglucosyl–acylglycerol moiety at its non-polar end. The amphipathic nature of LTA allows the molecule simultaneously to interact with charged (hydrophilic) substances and non-charged (hydrophobic) substances. LTA can therefore form molecular bridges. In *Streptococcus pyogenes* LTA is anchored to one or more proteins on the surface of the bacterial cells and interacts through its lipid moiety with the fatty acid binding sites of fibronectin molecules deposited on mucosal epithelial cells (Beachey & Courtney, 1989). Courtney *et al.* (1992) have recently demonstrated that two streptococcal adhesins, LTA and so-called M proteins, are involved in the adherence of group A streptococci to pharyngeal and buccal epithelial cells. Staphylococcal binding to mesothelial cells may involve the combined effects of LTA, teichoic acid and protein A (Haagen *et al.* 1990).

MUCIN AND BACTERIAL ADHESION

Many commensal and pathogenic bacteria have been found to colonize the intestinal mucus (Neutra & Forstner, 1987; Wanke *et al.* 1990). Whether such interactions favour the bacteria or the host continues to be the subject of much speculation. For many enteropathogens the initial contact by bacteria after entry into the small intestine from the stomach is with the unstirred mucus layer of the intestinal lumen (Wanke *et al.* 1990). In these situations the mucus may function as a permeability barrier or retention zone for bacteria, thereby restricting their free access to the underlying mucosa. Conversely, the ability of bacteria to interact with intestinal mucins can be an important step in facilitating colonization of the intestinal tract. If they are able to bind strongly to components of the intestinal mucus layer, their clearance by the motile and abrasive forces of digestion may be delayed and colonization of the intestinal tract may be favoured. Moreover, if the rate

of bacterial growth in and penetration of the mucus barrier exceeds the rate at which this layer naturally turns over and is eliminated from the gut, then clearance will be further delayed, again favouring colonization of the gut (Pærregaard *et al.* 1991; Mantle & Husar, 1993; Mantle & Rombough, 1993).

One of the most extensively studied bacterial–mucus interactions concerns *Yersinia enterocolitica*, an enteroinvasive bacterium that causes gastroenteritis in man. Invading *Y. enterocolitica* are capable of binding to purified intestinal mucins from both humans and rabbits. The binding appears to be plasmid mediated and involves mucin Gal and GalNAc residues (Mantle & Husar, 1993). Data from the same laboratories have shown that *Y. enterocolitica* is capable of degrading small intestinal and colonic mucins. The major enzyme involved may be a plasmid encoded or plasmid regulated proteinase the activity of which may be important in the establishment of infection by 'dissolving' the normal protective mucus barrier and allowing the organism to gain access to the underlying mucosa (Mantle & Rombough, 1993).

Specific receptors for several ETEC fimbrial adhesins have been identified in intestinal mucus. Pig small intestinal mucus has receptors to K88 and 987P fimbriated *E. coli* (Dean *et al.* 1989; Conway *et al.* 1990; Metcalfe *et al.* 1991). Calf small intestinal mucus contains receptors for K99 and F41 fimbriae (Laux *et al.* 1986; Mouricout, 1991), while rabbit ileal mucus has been shown to contain receptors for specific *E. coli* RDEC-1 AF/R1 fimbriae (Drumm *et al.* 1988). The role of these receptors in infectious processes is unclear. As discussed, receptors for K88 ETEC fimbrial adhesins are present on the microvillar membranes of susceptible pig phenotypes irrespective of their age (Willemsen & de Graaf, 1992). Mucus receptors, on the other hand, are absent in adult animals (Willemsen & de Graaf, 1992). However, it has been demonstrated that K88 receptor levels transiently increase in the late preweaning period. Conway *et al.* (1990) suggested that these 5-week pigs were less susceptible to K88 infection because of the high concentration of K88 specific receptor present in their ileal mucus which restricted access of the bacteria to the underlying epithelial cell membranes.

Further research is needed to identify the chemical composition and true origins of mucin glycoproteins extracted from intestinal mucus samples. Although many of these proteins may originate from goblet cells it is equally possible that many are microvillar membrane glycoproteins that have been released into the intestinal lumen. Recent *in vitro* adherence studies employing K88 fimbriae as cytochemical probes on histological sections failed to show receptors in goblet cell mucins (T. P. King & D. Kelly, unpublished observations). It is possible that at least some of the mucus receptor glycoconjugates seen in high numbers in preweaned pigs (Conway *et al.* 1990) are derived from maternal milk or are released from sloughed epithelial cells. Blomberg & Conway (1989) observed that K88 ETEC adhered more poorly to ileal mucus from piglets in which the gastric tissue was densely colonized by lactobacilli compared with mucus from piglets with sparsely colonized gastric tissue. Recent *in vitro* investigations have shown that *Lactobacillus fermentum* releases into the culture supernatant one or more proteinaceous components which affect mucus so that adhesion of cells bearing K88ab and K88ac is reduced (Blomberg *et al.* 1993).

As discussed above, there is conflicting evidence concerning the role of intestinal mucus in the aetiology of enteric infections. Whatever the effect of mucus on the gut ecosystem, it is likely that dietary factors which either limit the production of mucin, alter its composition, or enhance its degradation have the potential to influence colonization of the intestine by commensal and pathogenic bacteria (Neutra & Forstner, 1987; Wanke *et al.* 1990).

BACTERIAL GLYCOSIDASES

After their synthesis intestinal glycoconjugates may also be altered by the action of the endo- and exo-glycosidases of the bacteria themselves. Although modification of oligosaccharides of the epithelial surface itself has not been reported, there is considerable evidence that bacterial enzymes may hydrolyse the same glycosidic configurations in mucins. Following occlusion of ileal reservoirs in experimental dogs, bacterial counts increased 3-fold and glycosidase activities 5-fold; chronic inflammation of the mucosae and the abolition of blood group epitopes were attributed to degradation of protective mucin by the agency of bacterial glycosidases (Ruseler-van Embden *et al.* 1992). *Shigella flexneri* was shown to possess blood group B degrading activity (Prizont, 1982), while strains of *Ruminococcus* were able to carry out extensive hydrolysis of A/H or B substances in hog gastric mucin and some bifidobacteria were equally active but lacked A degrading activity (Hoskins *et al.* 1985). Significantly, much of the activity was extracellular, implying that attack of glycosyl moieties located on membranes would be feasible with consequent creation or abolition of specific bacterial adhesion sites.

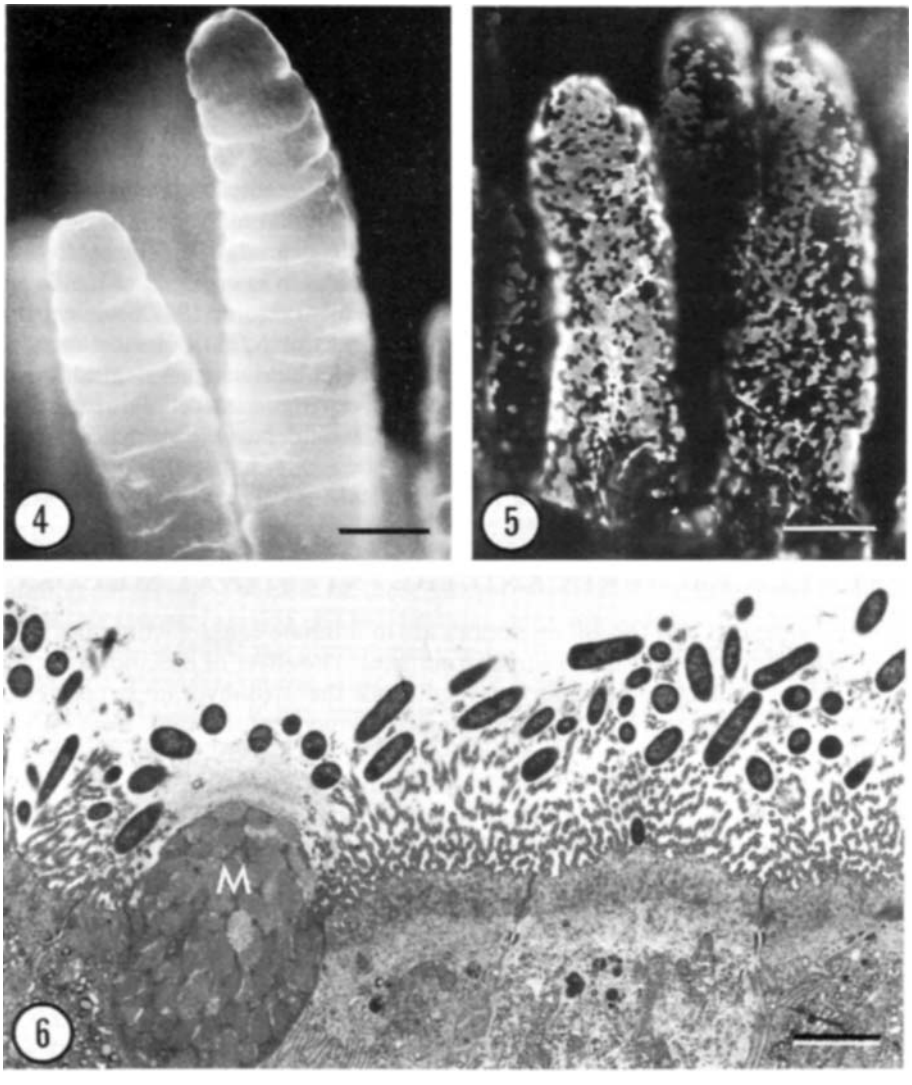
NUTRITIONAL INFLUENCES ON THE INTERACTIONS BETWEEN BACTERIA AND THE INTESTINAL MUCOSA

Components of the diet and the gut microflora are in intimate contact within the intestinal tract both in the lumen and at the absorptive surfaces. The effect of diet, however, may be direct or indirect. Dietary composition may influence the carbohydrate structures of the mucosal and mucin glycoconjugates with marked consequences for the adherent microflora. In the neonatal tract, exposure to components of maternal colostrum and milk may promote the selective growth of particular bacterial species. There is also good evidence for claims that the composition of adult diets may affect the bacterial ecology of the gut. Such effects offer potential routes whereby the gastrointestinal microflora may be manipulated for the benefit of the host.

DIETARY MODULATION OF BACTERIAL RECEPTORS IN THE NEONATE

As described in the previous sections, there is an increasing appreciation of the detailed carbohydrate structures constituting the receptor sites for adherent intestinal bacteria. The complex oligosaccharides involved are associated with proteins and/or lipids located in the mucosal membranes or with the secreted gastrointestinal glycoconjugates including mucins. It is conceivable that particular glycosyl structures appearing transiently represent 'windows of opportunity' for infection by enteropathogens whose adhesins exhibit the appropriate specificity, and such a rationale may explain, in part, why diarrhoea attributable to some species is particularly prevalent at certain stages of development.

During early development of the neonate, temporal expression of the glycosyl structures of the mucosal surfaces of the gut appears to be under endogenous control but may also be influenced markedly by diet (Kelly *et al.* 1992). Turck *et al.* (1993) found that the fucose, glucosamine and sulphate contents of mucin were increased in maternally fed piglets compared to their artificially fed counterparts. The authors suggested that breast fed animals may have a more effective mucus barrier against infection. Manipulation of the suckling regime has been shown (Figs 4 and 5) to alter the ontogenic expression of sialylated and fucosylated glycoconjugates (Kelly & King, 1991; Kelly *et al.* 1993). It is very



Figs 4 & 5. FITC-conjugated lectin from *Sambucus nigra* (SNA) used to label α2.6 NeuAc-Gal/GalNAc moieties on the villus surfaces of colostrum deprived (4) and colostrum fed (5) pigs at 1 week post partum. Postnatal reduction in epithelial sialylation, seen here as a mosaic of SNA + and SNA − cells, is more extensive in the colostrum fed animals. bars = 50 μm.

Fig. 6. Transmission electron micrograph showing coliform bacterial overgrowth associated with extensively disrupted intestinal microvilli in a rat fed on a diet containing kidney bean lectin. M = goblet cell mucin, bar = 2 μm.

likely that the highly sialylated intestinal epithelial surface in the neonatal intestine during the first days of life influences the establishment of the enteric flora. Attachment sites for several organisms may be masked by sialic acids whereas other pathogenic and non-pathogenic bacterial strains secrete sialidase enzymes which enable them to overcome host defensive mechanisms and create novel binding sites for colonization (Corfield, 1992). As already discussed, other enteric bacteria such as K99 ETEC opportunistically bind to

sialylated receptors and cause diarrhoea in neonatal but not adult pigs. The modulatory role of diet on temporal glycosylation changes in the neonatal intestine and its impact on bacterial colonization is a new and important subject for investigation.

Probiosis

Dietary inclusion of antimicrobials, particularly antibiotics, is established as an effective means of growth promotion in farm animals. Concern regarding the predicted emergence of resistant strains of pathogenic species has, however, provoked an increasing public rejection of this farming practice and has stimulated a demand for agents controlling growth and disease which are more 'natural' in concept. The rapid implantation of a commensal flora, coupled with the observation that germ free or antibiotic treated animals generally exhibit reduced resistance to invasion by enteropathogens, has given rise to the concept of protection by probiotics, i.e. 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance' (Fuller, 1992). The probiotic principle was originally propounded by Metchnikoff (1907) when he claimed that the putrefactive actions of lower gut species could be alleviated by consumption of fermented milk. From the latter he was able to isolate a bacillus, probably *Lactobacillus delbrueckii* ssp. *bulgaricus* which, with *Streptococcus salivarius* ssp. *thermophilus*, is used in yoghurt production. More recently, the survival of these species at stomach pH has been questioned (Conway *et al.* 1987) and efforts to devise effective probiotic preparations have focused on the use of species or strains which are known to be acid resistant and active gut colonizers (Kleeman & Klaenhammer, 1982; Mayra-Makinen *et al.* 1983). Probiotic preparations for human use have generally taken the form of fermented dairy products (yogurt, bifidus milk, kefir, etc.) and claims have been made for their efficacy in the treatment of various disorders including intestinal infection, hepatic encephalopathy, hypercholesterolaemia and carcinogenesis. The species most frequently associated with positive responses is *L. acidophilus*, although a strain of *Lactobacillus* termed GG and a non-pathogenic yeast, *Saccharomyces boulardii*, have both been effective in controlling recurring diarrhoea caused by *Clostridium difficile* toxin after antibiotic therapy (Gorbach, 1990; Surawicz *et al.* 1989). The consumption of bifidobacteria in a variety of fermented milk products has recently been popularized, particularly in Japan, where the fermentation industries are well developed. Growth of bifids in the gut may be stimulated by direct inoculation of viable cells, by the incorporation of bifidus factors such as the synthetic disaccharide lactulose or, for maximum effect, a combination of these strategies. When lactulose (3 g/d) was administered to healthy adults, 6-fold increases in excreted bifidobacteria were observed in 1–2 weeks and this correlated with significant decreases in ammonia and putrefactive products and with a reduction in faecal pH (Terada *et al.* 1992).

A rather wider range of species has been evaluated for probiotic efficacy in animals and, indeed, many of the preparations marketed by commercial concerns contain a number of bacterial species together with other factors held to be beneficial. A comprehensive discussion of animal probiotics is outwith the scope of this review but the subject has been addressed for several agriculturally important species by Fuller (1992).

The mechanisms underlying probiotic action remain somewhat obscure but suggested actions include competitive exclusion of enteropathogens, production of bacterocins etc., the production of hydrogen peroxide, liberation of free bile acids and suppression of ammonia and toxic amines.

Milk oligosaccharides as probiotics

The beneficial effects of breast feeding in averting gastrointestinal infections are undisputed (Kovar *et al.* 1984) and have been attributed to the separate or synergic

antibacterial action of factors which include secretory immunoglobulins, especially slgA (immune exclusion), lactoferrin (iron sequestration), lysozyme (cell wall lysis), lymphocytes and phagocytes. This list must be extended to include the oligosaccharides of human breast milk which may fulfil a directly protective function in addition to their role as bifidus factors (see below). The non-immunoglobulin fraction of milk was shown to be an effective inhibitor of the adhesion of *Vibrio cholerae* biotypes to human or chick erythrocytes (Holmgren *et al.* 1983); inhibitory activity was attributed to both free oligosaccharides and glycoproteins. Haemagglutination by CFA/I- and CFA/II-fimbriated *E. coli* was inhibited by glycolipids in human milk (Holmgren *et al.* 1983). The same group have also shown that the trace amounts of free GM1 ganglioside present in human milk are sufficient to suppress the *in vivo* enterotoxic effects of cholera toxin and the heat labile *E. coli* toxin (Kolstø Otnæss *et al.* 1983), both of which are known to recognize GM1 receptors in the intestinal membranes (Schengrund & Ringler, 1989). Perhaps the most interesting observation to date is the inhibition of adherence of human strains of *E. coli* (CFA/I- and CFA/II-fimbriated) to guineapig small intestine by the non-immunoglobulin fraction from human milk; significantly, no inhibition of type-1 fimbriated *E. coli* was noted (Ashkenazi & Mirelman, 1987).

Breast milk and bifidobacteria

In the human infant, breast milk promotes the establishment of the genus *Bifidobacterium*, contrasting with the response to formula feeding where bifidobacteria were either absent or part of a more complex anaerobic flora including *Bacteroides* and *Plectridium* (Moreau *et al.* 1986; Balmer & Wharton, 1989). Notwithstanding the disagreement which exists in the literature regarding the relative effects of maternal milk and milk replacers both in the human (Simhon *et al.* 1982; Benno *et al.* 1984) and in the pig (Ducluzeau, 1983), bifidobacteria are a common constituent of the faecal microflora of the neonate one week after birth. Differences in the species composition of the bifidobacterial population in the human infant intestine have also been widely reported (comprehensively reviewed by Bezkorovainy, 1989), although it must be acknowledged that some of the discrepancy may be attributable to the shortcomings of the identification methods used.

Various arguments have been advanced to account for the prevalence of bifidobacteria in the large intestine of the breast fed infant. It has been suggested that bifidobacteria are able to sequester iron, thus depriving enteropathogens of an essential virulence factor (Bezkorovainy *et al.* 1986) or that the lower buffering capacity of human milk compared to that of formula preparations based on cows' milk permits a lower gut pH with consequent inhibitory action on enteropathogens (Willis *et al.* 1973); this hypothesis has been disputed (Rose, 1984). An alternative concept, based on the existence of growth factors present in human milk (György *et al.* 1954*a*, *b*), has been more reliably validated.

In a more recent study (Beerens *et al.* 1980), specific *in vitro* growth factors for *B. bifidum* were found in human milk but were absent from the milk of other species including cow, sheep, horse and pig, although these latter did promote the growth of *B. infantis* and *B. longum*. Human blood group substances (Springer *et al.* 1954) and pig gastric mucin (György *et al.* 1954*a*) were also found to be very active. Carbohydrate-rich glycoproteins from both human colostrum (Nichols *et al.* 1975) and milk are able to promote the growth of *B. bifidum* var. *pennsylvanicus* (Bezkorovainy & Nichols, 1976) and *B. infantis* (Azuma *et al.* 1984); fragments with similar composition may be released from human casein by tryptic and chymotryptic digestion (Bezkorovainy *et al.* 1979) or by β-elimination (Bezkorovainy & Topouzian, 1981). Digestion products from bovine casein also demonstrate some activity (Kehagias *et al.* 1977).

Much effort has been invested in attempting to determine the nature and mode of action of the human milk factors. A common feature of the growth promoting oligosaccharides present in human milk is the presence of an internal β-*N*-acetyl-D-glucosaminide residue and, indeed, it has been shown that methyl β-*N*-acetylglucosaminide (but not the α-methyl anomer) has some activity (Rose *et al.* 1984). In accord with this indicated anomeric specificity, *N*-acetyl-D-glucosamine is some 10-fold less active but 4-*O*-β-D-galactosyl-*N*-acetylglucosamine, isolated from pig gastric mucin (Zilliken *et al.* 1955), is 3–5 times more active, suggesting that more complex structural factors are important for activity.

DIETARY MODULATION OF BACTERIAL RECEPTORS IN THE ADULT

Generally, the most disruptive dietary change which the young animal experiences is that of weaning. This is a gradual process under natural conditions but it is now common practice to wean abruptly to comply with the demands of modern pig production (Fowler, 1985) and rapid adjustment to a radically different diet is required by both animal and microflora. The 'stress' incurred, including the withdrawal of maternal antibodies and other protective factors in sow's milk, may render the host vulnerable to infection by opportunistic and other pathogens. In addition, intestinal glycosylation changes associated with weaning may have an important influence on bacterial colonization. Diminished α2,6 sialylation and enhanced expression of histo-blood group O and A antigens on both mucin and membrane glycoconjugates have been observed in the postweaned porcine small intestine (T. P. King, R. Begbie & D. Kelly, unpublished observations). Similar post-weaning increases in fucosylated glycoconjugates have been observed in the rat intestine (Taatjes & Roth, 1990). Biol *et al.* (1992) concluded that an increase in the intestinal fucosyltransferase activity that follows weaning in rats is largely induced by the weaning process.

Intestinal glycosylation processes are sensitive to nutritional factors (Biol *et al.* 1992; Kelly *et al.* 1992). The synthesis of the internal core of *N*-linked glycans is regulated by diet-induced variations at the phosphoryldolichol level, whereas modulation of the biosynthesis of the external regions of *N*-glycans or the biosynthesis of *O*-linked moieties is controlled by variations in systems transferring fucose, galactose, sialic acid and hexosamines (Biol *et al.* 1992). The influence of dietary protein levels on intestinal glycosyltransferase activities in the rat intestine was investigated by Martin *et al.* (1989). Fucosyltransferase was increased when animals were given a high protein diet but was unaffected by a low protein diet. *N*-acetylgalactosaminyltransferase and sialyltransferase were not modified by the high protein diet, but were decreased by the low protein diet. There is some evidence that protein quality influences intestinal glycosylation (Biol *et al.* 1990), but this area requires further investigation. Decreased intestinal fucosyltransferase activity has been observed in rats fed on diets supplemented with saturated or unsaturated fats (Biol *et al.* 1981). Dietary fats do not modify the kinetic parameters or the isoenzymic pattern of the intestinal soluble fucosyltransferase but fatty acids derived from diets may exert a direct effect on its activity (Biol *et al.* 1992). It has been suggested that membrane fluidity and Golgi function may be altered by dietary fats (Biol *et al.* 1992). The ingestion of carbohydrate-rich diets was found to have no influence on intestinal galactosyltransferase, *N*-acetylgalactosyltransferase or sialyltransferase activities, but it slightly depressed fucosyltransferase activity in the intestine in rats (Biol *et al.* 1981).

The capacity of micronutrients such as retinoic acid, the active metabolite of vitamin A, to modulate growth and differentiation of normal and transformed cells is well established (Glass *et al.* 1991; Labarriere *et al.* 1993). In several cell types, changes in glycosyltransferase

activities have been reported after retinoic acid treatment (Cummings & Mattox, 1988; Amos *et al.* 1990). Hypo- and hyper-vitaminosis A can significantly influence sugar-nucleotide availability and the initial stages of *N*-linked glycosylation of proteins (Biol *et al.* 1992). The influence of dietary vitamin levels on core glycosylation events and on the cellular systems transferring fucose, galactose, sialic acid, *N*-acetylglucosamine and *N*-acetylgalactosamine requires further study. There is evidence to suggest that there may be a relationship between dietary vitamin A and intestinal colonization by bacteria. In children deficient in vitamin A, increased rates of bacterial infection in the intestine have been observed (Gabriel *et al.* 1990). Vitamin A deficiency in rats is associated with the increased ability of *Salmonella typhimurium*, expressing type-1 mannose sensitive fimbriae, to adhere to proximal small intestinal enterocytes (Gabriel *et al.* 1990). Clearly, the role of minor dietary constituents as regulators of intestinal glycosylation and modulators of enteric health also deserves greater attention.

Dietary lectin toxicity and bacterial overgrowth

The relatively high protein and fibre contents of legume seeds make them attractive as feed and food components. The antinutritional effects associated with some seed components, however, have long been recognized by nutritionists and feed compounders, and it is customary to avoid problem seeds or to devise strategies such as heat treatment to inactivate the factors. Principal among these factors are lectins, which may be defined as 'proteins of non-immunoglobulin nature capable of specific recognition of, and reversible binding to, carbohydrate moieties of complex carbohydrates without altering the covalent structure of any of the recognised ligands' (Kocourek & Horejsi, 1983). Lectins are ubiquitous components of a wide range of commonly ingested foods (Nachbar & Oppenheim, 1980; Gibbons & Dankers, 1981) and may, in some circumstances, survive moderate processing treatments. Discussion of the wide range of physiological consequences of dietary lectin ingestion is outwith the scope of this review (for a recent review, see Pusztai, 1993); however, a particular feature, the induction of bacterial overgrowth, is relevant here and provides a useful model for discussion. Dramatic increases in the numbers of coliforms and other species have been consistently observed in the small intestines of conventional rats (Fig. 6; Wilson *et al.* 1980; Banwell *et al.* 1983, 1985), pigs (King *et al.* 1983), chicks (Untawale *et al.* 1978) and quail (Jayne-Williams & Hewitt, 1972) consuming diets containing kidney bean lectins (phytohaemagglutinins). The implication of bacterial overgrowth as a contributory factor in lectin mediated toxicity follows from the observation that germ free (Jayne-Williams & Hewitt, 1972; Rattray *et al.* 1974) or antibiotic treated animals (Banwell *et al.* 1983) were much less affected than their conventional counterparts. The nature of the involvement of phytohaemagglutinins in the dose dependent induction of a coliform-rich overgrowth which appears to be largely adherent to the mucosal surface (Banwell *et al.* 1985; Pusztai *et al.* 1993) has been the subject of much discussion (reviewed in Pusztai *et al.* 1990). The mucosal surface of the small intestine is normally sparsely populated with glycoconjugates carrying α-D-mannoside terminal residues (Pusztai *et al.* 1993; R. Begbie, unpublished) which are essential components of the adhesion sites for common type-1 fimbriated *E. coli* and other coliforms (Ofek & Sharon, 1990) and, to accommodate increased numbers of the latter, it is necessary to postulate the formation of novel sites. It has been suggested that these may be found in the form of the high mannose carbohydrate moieties of surface bound phytohaemagglutinins (King *et al.* 1983), but it has been claimed in a recent study in rats that the accelerated enterocyte turnover, stimulated by phytohaemagglutinin feeding, results in incomplete glycosylation of the brush border membrane glycoconjugates and consequent enrichment of polymannosylated glycan structures (Pusztai *et al.* 1993). Although

proliferation of enterotoxigenic species has not been reported in this study it may be speculated that augmentation of mannose-rich receptor sites would predispose the intestine to colonization by pathogenic members of the Enterobacteriaceae such as *Salmonella*, *Shigella* and *Klebsiella* which also carry type-1 fimbriae (Duguid & Old, 1980; Ofek & Sharon, 1990). In this respect, a superficial analogy with the phytohaemagglutinin model may be found in reports of an increased *Salmonella typhimurium* population of the caeca of chickens infected with the coccidian parasite *Eimeria tenella* (Baba *et al.* 1993); increases in staining with the mannose specific lectins, concanavalin A and *Lens culinaris* agglutinin, suggested that the density of adhesion sites on the mucosal surface had somehow been increased by the coccidial infection.

Malnutrition and bacterial translocation

Although the gut can tolerate and indeed benefit from a threshold level of indigenous adhering bacteria (Fuller, 1992) it would be wrong to assume that the indigenous flora is always benign. Under conditions of nutritional or other physiological stress the indigenous population may exceed a safe threshold and constitute a pathological burden (King *et al.* 1983; Spitz *et al.* 1994).

Malnutrition and long term parenteral nutrition have been shown to alter intestinal morphology, impair immune function and induce bacterial overgrowth. In the extreme, these changes have been implicated in the pathophysiology of translocation of bacteria leading to sepsis (Langkamp-Henken *et al.* 1992; Deitch, 1994; Silk & Grimble, 1994; Spitz *et al.* 1994). Surface proteins (invasins, internalins) implicated in the process of cellular invasion and bacterial translocation are expressed by several enteric bacteria including *Shigella*, *Yersinia* and *Salmonella* (Wick *et al.* 1991). Furthermore, it has recently been demonstrated that ETEC which normally colonize the mucosal surface possess specialized features which facilitate cellular invasion (Elsinghorst & Kopecko, 1992). It has been suggested that under conditions of environmental stress such invasive gene products are expressed in a wide variety of normally non-invasive organisms. An important research goal of the future is to elucidate the mechanisms by which both adherent and non-adherent bacteria become invasive (Boedeker, 1994). The invasion processes are complex and involve the interaction of bacteria with extracellular matrix components and cytoskeletal elements and plagiarization of normal signal transduction mechanisms of the intestinal epithelium (Wick *et al.* 1991). Dietary management is important for the prevention of colonization of the gut by enteropathogens but also has a protective role in the prevention of transepithelial translocation of bacteria (Deitch, 1994).

Chemical probiosis

Many of the more successful enteric organisms have developed strategies to resist displacement from the gut milieu by the digestive flow and, *inter alia*, the development of anchoring adhesive fimbriae (pili) represents a common feature of both commensal and pathogenic gut microflora (Ofek & Sharon, 1990). In seeking to elucidate the carbohydrate specificity of particular adhesins authors have generally employed *in vitro* competition assays with authenticated oligosaccharide structures (Firon *et al.* 1983, 1984; Leffler & Svanborg-Edén, 1986; Lindahl *et al.* 1987; Van Driessche *et al.* 1989) or with lectins of known specificity (Sellwood, 1980; Neeser *et al.* 1989; Willemsen & de Graaf, 1992), and increasing appreciation of specific oligosaccharide structures targeted by enteropathogens has given rise to the notion of selective therapeutic or prophylactic exclusion of enteropathogens from the intestinal mucosae. Two related strategies have been encompassed in the concept of chemical probiosis (by analogy with microbial probiosis) promulgated by Pusztai *et al.* 1990: each is based on competition by molecular species.

In the first, a soluble, non-toxic lectin may be administered where its carbohydrate specificity is selected to compete for the glycosyl binding site of the fimbrial adhesin. This approach is exemplified by the use of GNA, the snowdrop lectin, which is highly specific for 1,3α-linked D-mannose units, to block the proliferation of type-1 fimbriated *E. coli* in rat small intestine induced by ingestion of phytohaemagglutinins (Pusztai *et al.* 1993). A daily dose of 40 mg GNA effectively eliminated the coliform overgrowth in rat small intestine. It is conceivable that other mannose specific lectins present in foodstuffs which are more regularly consumed (by humans) may also have beneficial effects and, in this context, lectins from garlic (Kaku *et al.* 1992), shallots (Mo *et al.* 1993) and BanLec-1 from bananas (Koshte *et al.* 1990) may hold particular interest. It may be appropriate to sound a cautionary note against the universal application of the concept in view of the reported enhancement of adherence of non-fimbriated *Salmonella typhimurium* to rat small intestine by concanavalin A (Abud *et al.* 1989); here the authors suggest that colonization may be promoted by the formation of lectin bridges between the bacterial and mucosal cell surfaces.

The alternative approach of masking the fimbrial adhesins with competing oligosaccharide structures is more widely documented. Early work on the specificity of the fimbrial adhesins served to establish that *in vitro* attachment of bacteria to cell surfaces could be effectively inhibited by mono- and oligo-saccharides of appropriate structure (reviewed in Duguid & Old, 1980; Sharon *et al.* 1981). Successful control of experimental (non-gut) infection by the *in vivo* administration of simple sugars or glycosides (Aronson *et al.* 1979; Andrade, 1980; Fader & Davis, 1980) has been reported; in these instances type-1, mannose sensitive fimbriae were involved. A similar strategy has been applied prophylactically with broiler chicks where administration of D-mannose (2·5% w/v) in the drinking water effectively reduced colonization by a *Salmonella typhimurium* challenging strain from 84% in controls to 30% in treated chicks and also depressed faecal counts by $> 99\%$ (Oyofo *et al.* 1988). High mannose glycopeptides from ovalbumin were potent inhibitors of adhesion of type-1 fimbriated *E. coli* to guineapig erythrocytes and human buccal cells (Neeser *et al.* 1986), while the activity of larger oligomannoside glycopeptides released from legume glycoproteins was increased after trimming by α-mannosidase. Such readily available structures may prove useful in inhibiting *in vivo* binding by mannose specific adhesins associated with type-1 fimbriated enteropathogens. It is a prerequisite that these competing analogues, and the more complex carbohydrate structures recognized by ETEC strains, resist gut degradation. Perhaps as a consequence of the importance of these structures in both the glycocalyx and intestinal mucin, the gut lumen appears not to be endowed with the necessary endogenous hydrolases and the prospects for dietary intervention are encouraging. Extrapolating from their observation that ileal mucus components protect against epithelial colonization by *E. coli* K88+, Conway *et al.* (1990) have suggested that it might be possible to extend the protection to an earlier age by incorporation of 'synthetic receptors' in the diet. In this respect, however, perhaps the most spectacular success claimed so far is the use of glycans derived by proteolysis of blood plasma glycoproteins to protect against lethal doses of K99 ETEC in colostrum deprived newborn calves (Mouricout *et al.* 1990). In this study, 3×250 mg doses of the glycan preparation administered orally at the first signs of diarrhoea were sufficient to cure an experimental infection induced by 10^{10} bacterial cells.

Several of the proteinaceous toxins associated with enteric pathogens such as *Vibrio cholerae* and *E. coli* also bind to intestinal carbohydrate sites and competition strategies have been envisaged as a means of combating their effects. The ganglioside, GM1, bound to medical charcoal, was shown to bind luminal *V. cholerae* fully and also to reduce purging in the early stages of the disease. Since the effect was transient, however, it was considered

that oral GM1 might be useful mainly for prophylaxis in high risk groups (Stoll *et al.* 1980).

SUMMARY

In recent years there has been a major expansion in research on the molecular interactions between bacteria and the intestinal epithelium. Available data suggest that bacterial adhesins, in common with many well characterized plant lectins, recognize a diverse array of intestinal glycoconjugates. Serendipity and chemical inhibition studies have combined with good effect to identify receptor structures recognized by several fimbrial lectins. The chemistry of fimbrial lectins is in its infancy and the possibility should not be overlooked that simple chemical modifications may produce receptor molecules with even greater affinity for pathogens than the so-called native receptors (Mouricout, 1991). Molecular genetics and X-ray crystallography are proving to be invaluable in the identification of novel fimbrial and non-fimbrial adhesins, their specific interactions with host cell receptors and their role in pathogenesis. This information is essential for the design of effective disease prevention strategies. Nutritional intervention leading to altered receptor expression and/or the direct application of natural and artificial oligosaccharide structures as chemical probiotics may have considerable potential for the prophylaxis and therapy of intestinal infections.

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