COLONIC MICROBIOTA, NUTRITION AND HEALTH

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Table of Contents

INTRODUCTION

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It is clear that diet fulfils a number of important human requirements. These include the provision of sufficient nutrients to meet the requirements of essential metabolic pathways, as well as the sensory (and social) values associated with eating. It is also evident that diet may control and modulate various body functions in a manner that can reduce the risk of certain diseases. This very broad view of nutrition has led to the development of foodstuffs with added "functionality".

Many different definitions of functional foods have arisen. Most of these complicate the simple issue that a functional food is merely a dietary ingredient(s) that can have positive properties above its normal nutritional value. Other terms used to describe such foods include vitafoods, nutraceuticals, pharmafoods, foods for specified health use, health foods, designer foods, etc. Despite some trepidation, the concept has recently attracted much interest through a vast number of articles in both the popular and scientific media.

There are five identifiable ways in which a foodstuff can have an improved function on human well-being:

1) Elimination of a component that causes a negative effect (*e.g.* an allergenic protein, toxin)
2) Increasing the concentration of a natural component to a more desirable level (*e.g.* fortification with a micronutrient above the recommended daily intake, but compatible with dietary guidelines for disease prevention)
3) Addition of a component for which beneficial effects have been demonstrated (*e.g.* non vitamin antioxidants)
4) Replacement of a component, usually a macronutrient, the intake of which is often excessive and causes deleterious effects (*e.g.* fats), by a component that is more benign (*e.g.* emulsified carbohydrates)
5) Improvement of the bioavailability of food components for which beneficial effects have been demonstrated.

The design and development of functional foods is a key nutritional issue which should rely on clear scientific guidelines. Some important aspects are given in Table 1.

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Table 1. Scientific aspects pertinent to functional food development

1. Basic scientific knowledge relevant to functions:
- sensitive to modulation by food components
- pivotal to the maintenance of well-being and health, including disease prevention
2. Exploitation of this knowledge in the development of markers key to relevant functions
3. The generation of new hypothesis-driven human intervention studies which:
- include the use of validated, relevant markers
- allow the establishment of effective and safe intakes
4. Development, and exploitation, of advanced technologies for efficacious human studies:
- minimally invasive
- applicable on a large scale, including multiple centres

The first generation of functional foods largely involved the addition of certain components (*e.g.* vitamins, micronutrients) to the diet. However, one of the most promising current targets for functional food development is the gastrointestinal tract (GIT). The large intestine is, by far, the most densely populated areas of the human GIT. The organ is involved with control of transit time, bowel habit, absorptive and mucosal function - all of which may be associated with the resident gut microflora. Moreover, it appears that the resident microbiota may, through its normal metabolic state, exert other important physiological processes relevant to host health and disease. These are discussed in many of the following chapters.

The purpose of this book is to overview current knowledge of the activities and functions of the gut microflora. This is approached through the collation of recognised expertise in the areas of gut microbial function, molecular biology, clinical nutrition and industrial relevance. The following aspects of gastrointestinal microbiology are reviewed in terms of gut functionality:

- the GIT flora composition and activities
- the fermentation process
- luminal and biofilm bacterial processes
- gut flora modulation through diet (probiotics, prebiotics)
- the harnessing of molecular methodologies in gut microbiology
- applied relevance in terms of health ourcome
- consumer perspectives

It is implicit that the impact of gastrointestinal microorganisms on this contemporary area of nutritional sciences has much relevance.

CHAPTER 1

The Human Colonic Microbiota

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1 Introduction

It has become increasingly evident over the last 20 years that the large intestine is a highly specialised digestive organ, which through the activities of its constituent microflora, rivals the liver in metabolic capacity, and in the diversity of its biochemical transformations. Approximately 90% of the 10^{14} cells associated with the human body are microorganisms, and the vast majority of these reside in the large bowel. The colonic microbiota consists of approximately 10^{13} cells which, with the possible exception of bacteria growing in the oral cavity, are physiologically very different to those associated with any other part of the host. Several hundred bacterial strains and species normally exist in the large intestine, with viable counts typically being in the region of 10^{12} per gram of intestinal contents. While host tissues and other substrates of endogenous origin (sloughed epithelial cells, mucins, pancreatic and other secretions) are continually being broken down and recycled by intestinal bacteria, the species composition and metabolic activities of the colonic microbiota are primarily determined by diet. Therefore, what we eat, particularly carbohydrate and protein, affects ecological, physiological and metabolic events in the large bowel. An outline of the main substrates available for intestinal bacteria is shown in Fig. 1. Through fermentation and the absorption and metabolism of short chain fatty acids (SCFA), the large intestinal microflora plays an important role in host digestive processes, enabling energy to be salvaged from unabsorbed dietary residues, as well as body tissues and secretions.

Intestinal microorganisms play a major role in health and disease, and affect human physiology in a multiplicity of ways (see Table 1), through obligate host requirements for bacterial fermentation products, maintenance of colonisation resistance to microbial pathogens, activation or destruction of genotoxins and mutagens, and modulation of immune system function. For example, the colonic microflora affects the cytokine network that regulates the effector arms of the immune response, as evidenced by the fact that reduction in IFN-γ and IFN-α production in aging mice is reversed with

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lactic acid bacteria probiotics (Muscettola *et al.*, 1994), while in human peripheral blood mononuclear cells, these organisms have been shown to induce formation of IL-1ß, TNF-α and IFN-γ, though not IFN-α or IL-2 (Pereyra and Lemmonier, 1993).

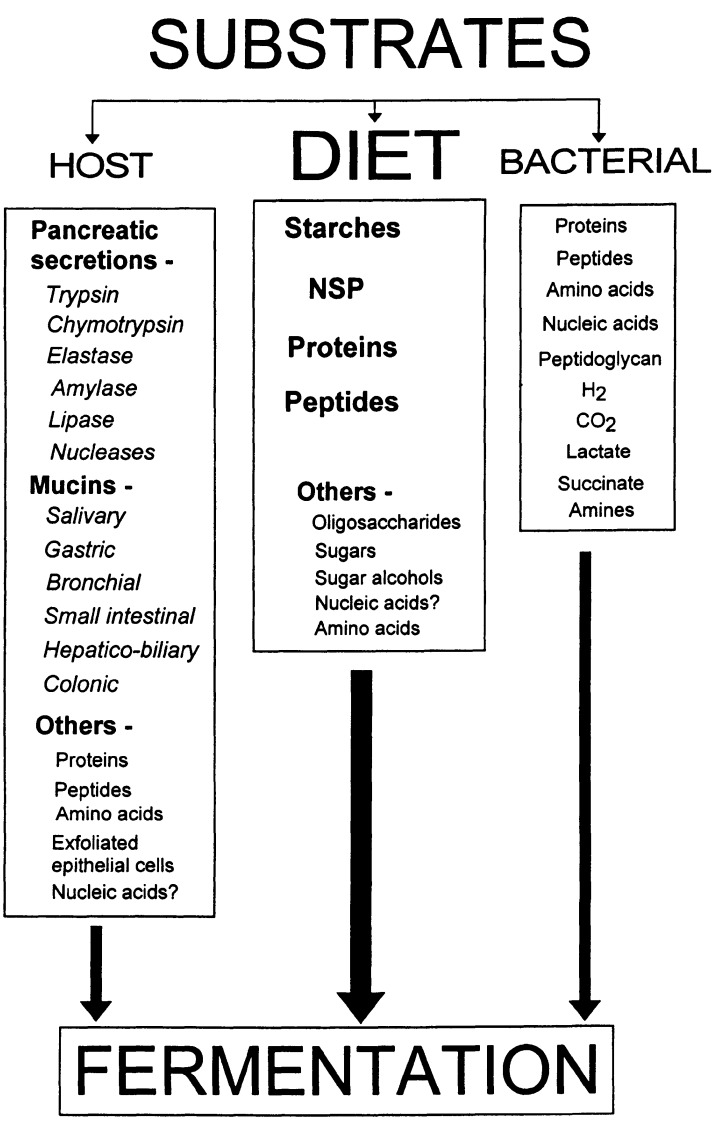

Figure 1. Substrates available for fermentation by bacteria growing in the large intestine. (NSP = non starch polysaccharide)

Table 1. Some health-related activities associated with bacteria growing in the large intestine

Processes	Examples	Effects on host
Carbohydrate fermentation	Digestion of starches, non-starch polysaccharides and oligosaccharides that escape digestion in the small bowel	SCFA formation supports epithelial cell growth, energy reclamation and other aspects of large bowel function. Increased bacterial growth and faecal output. Reduced absorption of toxic products of protein fermentation. Gas production
Proteolysis and amino acid fermentation	Recycling of C and N in proteins and peptides in dietary residues and pancreatic secretions. Production of ammonia, amines, HS^-, thiols, phenols and indoles	Toxic metabolites associated with hepatic coma, other neurological symptoms, cytotoxicity and colon cancer. Gas production
Hydrogen disposal	Methane production, acetogenesis, HS^- formation	Reduction in colonic gas volume, HS^- cytotoxicity and ulcerative colitis?
Bile acid metabolism	Deconjugation and dehydroxylation of bile acids, desulphation of bile acid sulphates	Absorption of secondary bile acids. Possible promoting activity in colon cancer
Mutagen production	N-Nitrosation of secondary amines	Large bowel cancer
Metabolism of neutral steroids	Chemical modification of cholesterol, plant sterols and steroid hormones	Reabsorption and recycling of reduced corticosteroids, progesterone, oestrogens, role in breast cancer?
Transformations of xenobiotic substances	Desulphation of cyclamate to produce cyclohexylamine	Formation of acutely toxic substances, prolonged enteropathic circulation of foreign compounds
	Desulphation and deconjugation of drugs excreted in bile	
	β-Glucuronidase/methylazoxy-methanol formation from cycasin	
	Conversion of azo bond in sulfasalazine to produce the active drug 5-aminosalicylic acid	
Metabolism of lignans and phytooestrogens	Conversion to enterodiol, enterolactone and equol by the microbiota	Oestrogenic and antioestrogenic effects: Related to fertility and breast cancer
Immune system development and modulation	Shown in probiotic studies, investigations with gnotobiotic animals	Enhanced resistance to infection
Colonisation resistance	Barrier effect of mature microbiota against invading species. Degraded during illness or antibiotic/drug treatments	Resistance to disease

2 The human large intestine

Due to gastric acid and the washout effect resulting from rapid passage of digestive substances through the stomach and small bowel, the principal areas of permanent colonisation of the human gastrointestinal tract are the terminal ileum and large intestine. This is primarily a result of the slowing down of movement of digestive material in the colon, which allows time for a complex and stable microbial ecosystem to develop (Cummings, 1978).

In adults, the large intestine is approximately 1.5 m long, and has a volume in excess of 500 ml. It daily receives about 1.5 kg of material from the small gut, the majority of which is water and is rapidly absorbed. The colon contains around 200 g of contents, of which approximately half consists of bacterial cells (Cummings *et al.*, 1990). Microorganisms are therefore a major component of faeces, comprising approximately 55% of solids in persons consuming Western-style diets (Stephen and Cummings, 1980). In the United Kingdom, the average daily output of material is 100-200 g (Cummings *et al.*, 1992).

After digestive materials enter the caecum, rapid breakdown of readily fermentable substrates occurs. Dietary residues form a pool of digesta in the proximal colon, and there is a significant degree of mixing from one day to the next (Wiggins and Cummings, 1976). Portions of this material are periodically transferred to the transverse and distal colon, where further water absorption increases the viscosity and reduces mixing. Due to utilisation of digestive substances by bacteria in the proximal colon, particularly carbohydrates, there is a progressive reduction in substrate availability towards the distal gut, affecting such factors as the type and amounts of fermentation products that are formed, as well as colonic pH (Cummings *et al.*, 1987). This can influence a number of metabolic processes in the bowel, including fermentation product formation (Blackwood *et al.*, 1956) and enzyme activities such as bile acid 7-α-dehydrogenase (Midvedt and Norman, 1968), proteases and peptidases (Macfarlane and Macfarlane, 1997; Macfarlane *et al.*, 1992a). Thus, some bacterial populations and activities may be restricted to, or predominate in, particular parts of the large gut (Macfarlane *et al.*, 1992b).

Transit of digestive substances through the large intestine consists of two chronological components, time spent in the mixing region (caecum and ascending colon), and time spent in passage and storage of partly solidified stool in the distal bowel (Wiggins and Cummings, 1976). The length of time digestive material spends in the large intestine is an important determinant of colonic bacterial metabolism. Long colonic transits affect the metabolism of carbohydrates, proteins and xenobiotic substances, and have been linked to the occurrence of large bowel cancer (Cummings *et al.*, 1992). Moreover, a significant correlation exists between transit time and bacterial mass in the large intestine: Stephen *et al.* (1986) used the drug senokot to speed up gut transit time from 64 to 25h in human volunteers. This increased mean stool weight from 148 to 285 g/d, with bacterial mass increasing from 18.9 to 20.3 g. In contrast, treatment with codeine/lopramide slowed colonic transit times from 47 to 88h and reduced bacterial cell mass from 18.9 to 16.1 g.

3 Acquisition and development of the colonic microflora

Humans are born with sterile colons, but bacteria begin to appear in excreta over the first few days of life. Breast and bottle-fed infants are inoculated by large numbers of Gram positive and Gram negative microorganisms during birth (Bullen *et al.*, 1976), and an outline of events relating to subsequent colonisation events in the large bowel during the first 2 years is shown in Fig. 2. Facultative anaerobes such as enterococci and enterobacteria are early colonisers, and they reduce pO_2 sufficiently to enable anaerobic organisms, particularly bacteroides and bifidobacteria, to establish. After weaning, the intestinal microbiota becomes more stable, as adult-type climax bacterial communities develop.

Considerable discrepancies occur in the literature concerning developing bacterial populations in the infant colon, which probably reflect inter-individual, cultural and environmental differences, and possibly, the methodologies employed in their study. For example, the work of Simhon *et al.* (1982) indicated that the faecal microflora of breast-fed and bottle-fed infants was similar, but many other investigations show that marked differences exist between these two groups, and that bifidobacteria, in particular, are the predominant organisms in formula-fed babies.

As shown in Fig. 3, a study of 35 breast-fed and 35 bottle-fed Japanese children (Benno *et al.*, 1984), indicated that bifidobacteria occurred high numbers in both groups, though some other types of intestinal bacteria including anaerobic Gram positive cocci, eubacteria, lactobacilli, enterobacteria, enterococci and bacteroides occurred in significantly lower numbers in the breast-fed infants.

In some European children, *Bifidobacterium bifidum* seems to predominate in the colons of breast-fed children (see Fig. 4), due to the presence of specific growth factors in human milk. Infant formulas, cows, sheeps or pigs milk milk do not contain these substances, and do not promote growth of this species, although they can support *B. infantis* and *B. longum* (Beerens *et al.*, 1980). Recent work indicates that *B. longum, B. adolescentis, B. pseudocatenulatum and B. parabifidum* can also occur in high numbers in some breast-fed children (Kleessen *et al.*, 1995). In Japan, *B. breve*, and *B. infantis* have been reported to be the numerically important bifidobacteria in infant faeces (Mitsuoka, 1989).

A variety of monosaccharides are present in human milk including lactose, glycoproteins and glycolipids, sialic acid, fucose and N-acetylglucosamine. Milk also contains over one hundred other more complex carbohydrates, including oligosaccharides (degree of polymerisation, DP, 3-11) based on lactose, and containing N-acetylglucosamine, fucose, galactose, sialic acid and glucose (Miller *et al.*, 1994). These oligomers are not digested to a significant extent in the small bowel of young infants, which has led to the suggestion that they have a multi-functional role in the gut, acting as ligands for pathogenic microorganisms and preventing their adherence to the small bowel mucosa, while serving as carbon sources for bifidobacteria in the colon (Brand Miller *et al.*, 1995).