The Large Intestine in Nutrition and Disease

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Dr. John H. CUMMINGS

Course given in the frame of the *Chaire Danone* 1995
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Avant-propos

The Institut Danone is an association of scientists involved in human nutrition. Its mission is to forge links between the scientific community and health and education professionals. It was within this perspective that the Institut created the Danone Chair.

The aim of the Danone Chair is to diffuse recent findings in the field of human nutrition. Each year, one French-speaking university and one Dutch-speaking university from Belgium organise a course of lectures headed by a Belgian or foreign authority in the field. This course, aimed at a multi-disciplinary university audience, includes an introductory lecture followed by a 15 hour course. The full set of lectures is published by the Institut Danone as a monograph.

The Danone Chair was awarded during the 1994–1995 academic year to the Université Catholique de Louvain and the Katholieke Universiteit Leuven. Holders of the Chair were Dr. John Hedley CUMMINGS, associate lecturer in the Faculty of Medicine, University of Cambridge and member of the Scientific staff of the MRC DUNN Clinical Nutrition Centre, Cambridge (UK) and Prof. Paul ROZIN, Professor of Psychology at the University of Pennsylvania (USA).

The current monograph is based on the lectures given by Dr. CUMMINGS in the frame of the Danone Chair at the Université Catholique de Louvain.

The Institut Danone warmly thanks Dr. CUMMINGS for the quality of his lectures and his monograph. The Institut also thanks Professor Marcel ROBERFROID for his contribution to the organisation of the Chair and Mrs Micheline POPULER for her editorial work on the text of Dr. CUMMINGS'monograph.

Prof. Dr. Kenny DE MEIRLEIR
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of the Scientific Council

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of the Board of Directors

Foreword

Dr. John Hedley CUMMINGS, a born English gentleman, is presently a member of the Clinical Scientific Staff at the MRC DUNN Clinical Nutrition Centre and Honorary Consultant Physician at ADDENBROOKE's Hospital in Cambridge as well as Associate Lecturer in the Faculty of Clinical Medicine, University of Cambridge, UK.

He was trained both as a medical doctor and as a biochemist in the Universities of Leeds, London and Cambridge. He is an accredited specialist in Gastroenterology and Internal Medicine. His teaching at the University of Cambridge, London School of Hygiene and Tropical Medicine and King's College in London concerns mainly human nutrition.

The major contribution of J.H. CUMMINGS to human medicine is the recognition that the large bowel is playing key physiological roles not only with regard to water and ion absorption but also to carbohydrate and protein metabolism. Already his first scientific paper published in *Lancet* in 1973 concerned the "Role of colon in ileal-resection diarrhoea". Since then, he has published some 150 scientific papers which have contributed significantly to our present understanding of the key role of the colonic microbiota that colonises the large bowel creating a true symbiosis. His contribution is at the basic level of knowledge in the physiology of the colon, but also in the pathophysiology of important bowel diseases and finally in the methodologies which we all need to investigate these functions and their pathologic dysfunction.

He was amongst the first to recognize the importance for health of dietary carbohydrates which even though they are not digested in the upper part of the gastro-intestinal tract are extensively fermented in the colon by the anaerobic bacteria. The concepts of energy salvage, bifidogenesis, colonisation resistance are all concepts to which his research has contributed enormously.

His laboratory in Cambridge has been a place for postgraduate and post-doctoral training of a vast number of scientists from all over the world many of whom are actually back in their country where they occupy leading scientific positions.

Besides being a leading scientist, J.H. CUMMINGS is also known for the quality of his conferences and his lectures which are always very clear, well illustrated, well presented and very stimulating. These are the main reasons why he has been and still is invited worldwide to participate at the most important scientific meetings in the fields of nutrition and gastroenterology.

For those who have the privilege to know him personally, J.H. CUMMINGS is not only a top level scientist with whom it is so stimulating to discuss, but also a man of great humanism interested in art and open to all problems of mankind. He enjoys life and particularly good food as expected for someone interested in human nutrition !

It has been a great privilege and a great pleasure for me to welcome J.H. CUMMINGS as the first Danone Lecturer in the Faculty of Medicine at the Université Catholique de Louvain. His lectures have all been so rich and so stimulating. We are grateful to the Institut Danone for having created such a Chair which will, without any doubt, contribute significantly to the implementation of nutrition as an essential part of education in biomedical disciplines.

Prof. Dr. Marcel ROBERFROID
Pharmaceutical Sciences Department
Université Catholique de Louvain

Acknowledgements

The work described in this monograph has been done in Cambridge by members of the Medical Research Council Dunn Clinical Nutrition Centre – Gut Group, without whose ideas and help the concept of the human large bowel as a digestive organ would not have developed. I am especially glad to acknowledge the longstanding collaboration of Drs Sheila Bingham, Hans Englyst, George Macfarlane and Glen Gibson. In addition many other members of this group, past and present, have contributed including: Emily Beatty, Aedin Cassidy, Elaine Collard, Edna Collinson, Leonora Durcell, Geoff Hudson, Alex Loktionov, Sandra Macfarlane, Ian O'Neill, Max Pitcher, Mike Quigley, Shirley Runswick, Judith Wills, Philip James, Hugh Wiggins, Ian McNeil, Aiden Challen, Henrik Andersson, John Banwell, David Saunders, Alison Stephen, Ru Pomare, Wolfgang Scheppach, Stefan Christl, Ashok Chacko, Charles Etterlin, Laura Bravo, Catherine Michel and many students who have enlivened the group over the years.

The text of this monograph covers most of the lectures given during my tenure of the Danone Chair at UCL Brussels. It is drawn partly from previously published reviews and books to which the reader should refer for full information on these many subjects. Such reference sources include particularly *The Large Intestine* edited by Phillips *et al.* and published by Raven Press (1992); *Constipation*, by Kamm and Lennard-Jones, Wrightson Biomedical Publishing Ltd (1994); *Human Colonic Bacteria* by Gibson and Macfarlane, CRC Press (1995); *Diseases of the Gut and Pancreas* by Misiewicz *et al.*, Blackwell Scientific Publications (1994); *Physiological and Clinical Aspects of Short Chain Fatty Acid Metabolism* by Cummings *et al*., Cambridge University Press (1995).

Finally, I am most grateful to the Danone Institute of Belgium and particularly to Professor Marcel Roberfroid of UCL Brussels for making this visiting Professorship an enjoyable and productive exercise both scientifically and in the development of nutritional medicine in Europe.

Dr. John H. Cummings

Summary

The classical view of the human colon is that of an organ which absorbs salt and water and provides a mechanism for the orderly disposal of waste products of digestion. It is now clear, however, that it has a major role in digestion through the salvage of energy, and possibly nitrogen, from carbohydrate and protein not digested in the upper gut. This is achieved by the metabolism of anaerobic bacteria and is known as fermentation.

The principal dietary substrates for fermentation in man are starch, non-starch polysaccharides (NSP) which are the major plant cell wall carbohydrates, oligosaccharides and some sugars. These are broken down to short-chain fatty acids (SCFA) (acetate, propionate and butyrate), the gases H_2 and CO_2 and stimulate bacterial growth, or biomass. SCFA are rapidly absorbed, acetate reaches muscle where it serves as a fuel for the tissues, whilst propionate is largely cleared by the liver and may contribute to the regulation of lipid metabolism. Butyrate is metabolised by the colonic epithelium, which derives 60-70% of its energy from bacterial fermentation products. Butyrate is an important regulator of cell growth and differentation. Hydrogen disposal in the colon is either by excretion through the lung or in flatus, or through further metabolism to methane, sulphide and acetate. Stimulation of biomass leads to increased faecal bulk and bacterial protein synthesis.

The principal chronic colonic diseases, constipation, cancer, diverticular disease and ulcerative colitis, are also affected by fermentation and its products. Bowel habit is partly determined by the amount of carbohydrate reaching the colon, whilst in ulcerative colitis there is evidence for sulphur compunds and other bacterial end-products affecting cell metabolism.

Over the last decade, much progress has been made in our understanding of the pathology and molecular genetics of colon carcinogenesis. This has culminated in the development of a well-defined model for the aetiology of colon cancer. It has been proposed that the pathology of malignant tumours in the colon frequently (though not exclusively) follows a series of events known as the adenoma-carcinoma sequence. Certain genetic alterations are known to occur frequently in colonic adenocarcinomas. Fermentation in the large bowel is now understood to be a protective factor in colon cancer. The role of butyrate on colonic cells at the molecular level provides an exciting link between dietary factors and the development of colon cancer. Whilst the fermentation of NSP (fibre) undoubtedly generates butyrate, *in vitro* studies have shown that the fermentation of starch generates greater proportions of butyrate than the fermentation of other carbohydrates.

The recommendation for countries with Western-style diets to increase their intake of starch and NSP has become universal since it was first suggested by the US Senate Select Committee on Nutrition and Human Needs in 1977—the McGovern Report. Limits for starch intake have usually been set by default, that is starch is considered to be an energy filler in the diet once limits have been set for fat and protein.

The specific advantages of consuming starch are that as a substitute for saturated fat it will lower blood cholesterol, substituting for sugar will reduce the risk of dental caries and, together with resistant starch, may contribute to the prevention of bowel cancer. Moreover, many starchy foods such as cereals, pulses and vegetables contain a wide variety of micronutrients which sugary and fatty foods do not.

A number of national bodies have now made quantitative recommendations for increases in "dietary fibre" intake but, because of the lack of an agreed definition of fibre and of a method for measuring it, these recommendations do not cross international boundaries very well. Therefore, the BNF, WHO and the UK Department of Health have opted for NSP as the best index of fibre. However the evidence points to the goal of a diet *characterised by* high NSP (and starch) intakes.

1 – Introduction

How does the elephant grow and maintain its magnificent body on such an apparently poor diet of leaves and branches? Unlike the cow, sheep, antelope, camel and even the giraffe, it has no rumen in which to ferment a diet so resistant to normal digestive enzymes. However, the elephant, like the rat, horse, rabbit, zebra, rhinoceros and bear, has a highly developed caecum and large intestine which contain an abundant anaerobic bacterial flora, that takes over from where endogenous digestive enzymes have failed and allows the breakdown, or fermentation, of more resistant components of the diet. The contents of the caecum and large bowel of the elephant account for 12% of its weight [1] which, in a fully grown African elephant, will amount to 555 kg.

Human large bowel contents amount to only around 0.22 kg or 0.3% of our weight [2]. Nevertheless, in the vast chambers of the large bowel of the elephant, the human and even the minute colon of the shrew, fundamentally the same process goes on, namely fermentation.

The large intestine is a digestive organ in which anaerobic bacteria break down carbohydrates such as fibre and starch not digested in the upper gut. The principal products are short-chain fatty acids (SCFA), acetic, propionic and butyric acids, which are absorbed. SCFA provide energy for the host, which can be up to 30% of basal energy requirement in some species, although in the human on Western diets it is probably nearer 5% [3–5]. Fermentation also gives rise to hydrogen and carbon dioxide, which have to be excreted, and to biomass (bacteria) which in turn affects a number of metabolic processes in the colon. Biomass is excreted in faeces, hence the laxative properties of fermented carbohydrates.

Other substrates, particularly protein, are also fermented and produce a range of products in addition to SCFA (**Table I**).

Thus are found in large bowel contents biomass, epithelial cells, residual food particles and mucus, together with the gases nitrogen, hydrogen, carbon dioxide and methane. In addition acetate, propionate and butyrate are the principal anions, with smaller amounts of the branched-chain fatty acids (isobutyrate, isovalerate), and lactate, amines, phenols and sulphide. Cations are mainly potassium, calcium, magnesium, the ammonium ion and sodium, with plant sterols, and degradation products of cholesterol and bile acids making up the overall matrix.

Fermentation dominates large bowel function. It affects every process including salt and water absorption, pH, epithelial cell metabolism, motility and bowel habit and colonisation resistance, in addition to providing products which are absorbed and reach the liver and peripheral tissues. Humans are able to live

Table I — Major functions of the human large intestine[1].

Process	Products
Digestive	
Carbohydrate fermentation	Short-chain fatty acids H_2, CO_2, CH_4 Biomass
Protein breakdown and amino acid fermentation	Short-chain fatty acids Branched-chain fatty acids H_2, CO_2 Phenols Amines Ammonia
Absorptive	All the above except biomass Na, K, Cl, HCO_3, H_2O Bile acids
Excretory	Biomass Food residues Epithelial cells Mucus H_2O, H_2, CO_2, CH_4 Toxic waste
Hormonal	Neurotensin Enteroglucagon PYY Somatostatin
Synthetic	B and K vitamins
Storage	For control of defaecation
Metabolic (bacteria)	Bile acid dehydroxylation Sulphate ➜ sulphide Nitrate ➜ Nitrite ➜ Ammonia Many other hydrolytic and reductive reactions

[1] For general texts about the large intestine see [6-11].

satisfactorily without the large intestine [12] but most of us have to live with it and are prey to a number of serious diseases which it can develop (**Table II**).

Table II — Principal large bowel disorders.

• Acute infections	• Crohn's disease
• Cancer	• Constipation/Irritable bowel
• Diverticular disease	• Ano-rectal disorders
• Ulcerative colitis	• Appendicitis

For the majority of these diseases, especially large bowel cancer, inflammatory bowel disease and diverticular disease, we do not at present have a complete explanation of their origins. Understanding fermentation and its consequences may help to unravel their aetiology and, if so, provide a direct relation between diet and large bowel diseases.

2 – Anatomy, Bacteriology and Function

2-1 Anatomy

The human colon is not neatly laid out around the abdominal cavity but lies with loops and flexures throughout the pelvis and in varying configurations around the abdomen (**Figure 1**).

In a study of the large intestine of 46 sudden death victims from both Africa and the UK [2] the large bowel was 154 (range 113 to 207) cm in length and had a surface area of 1274 (731 to 2509) sq cm. The bowel contained 222(58-904) g of contents, of which 36 g was dry matter and 186 g water. Moisture content ranged from 85% in the caecum to 77% in the recto-sigmoid region. pH was 5.8 in the caecum, rising to 6.5 in the recto-sigmoid.

Mouth to anus transit through the human gut takes about 60 h in UK adults [13] with women 72 h and men 55 h. Of this 4–6 h will be mouth to caecum transit time so residence in the colon is around 54 h. Transit in Africans is reported to be much quicker—in the region of 24 to 48 h [14]. Transit through different regions of the colon has been measured using a variety of x-ray techniques. Reported times are 7–24 h for the caecum and right colon, 9–30 h for the left colon and 12–44 h for the recto-sigmoid [15].

The composition of large bowel contents has largely been derived from measurements of faeces but some data are also available from autopsy material. In the caecal region the largest fraction is proteinaceous at 40% (N x 6.25) of dry matter, followed by carbohydrate 20%, fats 20%, minerals 10% and residual moisture about 10%. What this means in practice is lots of bacteria sitting on food particles surrounded in a matrix of glycoprotein and exfoliated cells (**Figure 2**).

2-2 Bacteria

Apart from food residues, the major component of colonic contents is bacteria whose numbers exceed 10^{11} per g dry matter. Several hundred species have been identified [16–19] but some 30–40 species belonging to 5 or 6 genera account for 99% of biomass.

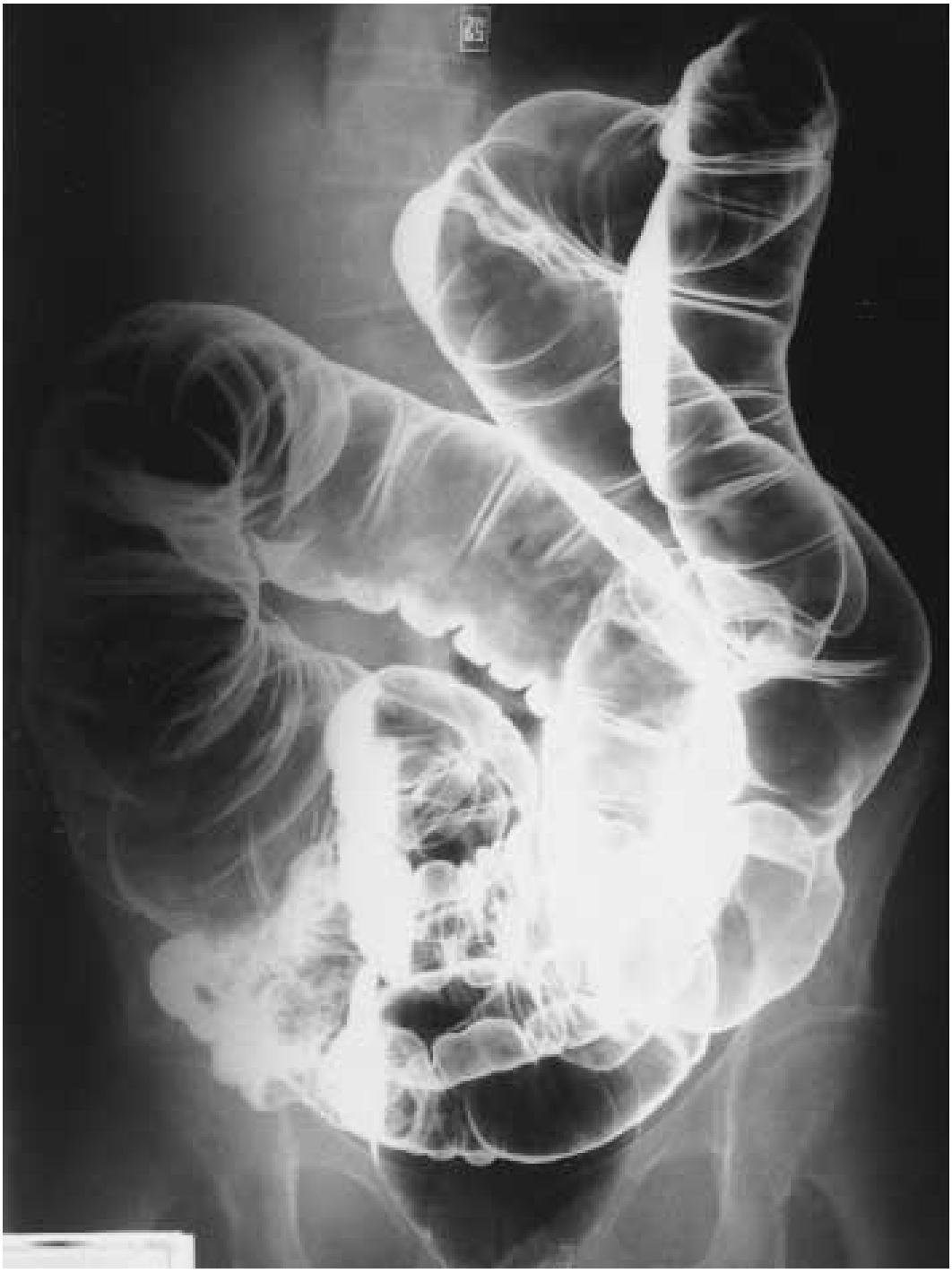


Figure 1 — Barium x-ray of the human large intestine. The bowel loops are filled with air and demonstrate one of many varying anatomical patterns which the large intestine takes (from Dr. Max FREEMAN, with permission).

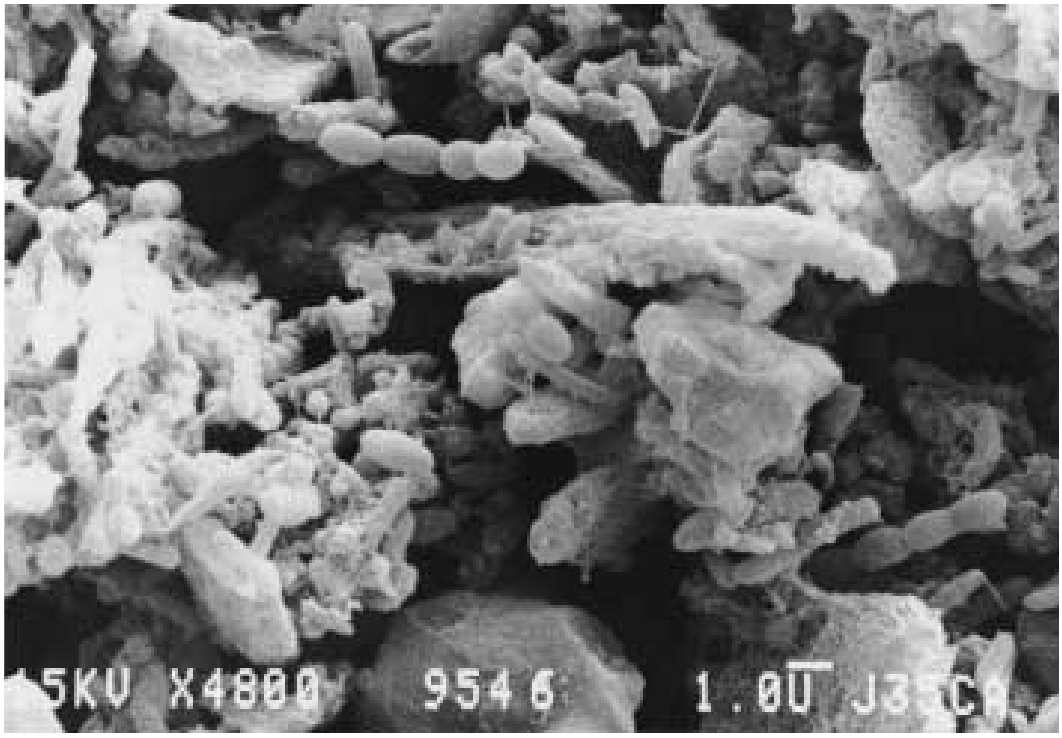


Figure 2 — Electron micrograph of human large intestinal contents, showing multiple forms of bacteria resident upon food particles (from the work of Dr. G.T. MACFARLANE, with permission).

Table III lists bacteria commonly isolated from the human colon. Bacterial counts of individual species range over several orders of magnitude, and the nutrition and metabolic products of different bacterial groups vary considerably. Most bacteria growing in the colon are non-sporing anaerobes and include members of the genera *Bacteroides, Bifidobacterium,* and *Eubacterium* among many others. Clostridia are also represented, though they are outnumbered by the non-sporing anaerobes, as are facultative anaerobes such as streptococci and enterobacteria. Quantitatively, the most important genera of intestinal bacteria in animals and man are the bacteroides and bifidobacteria, which can account for 30% and 25% of the total anaerobic counts respectively. Amongst the Gram-positive, non-sporing rods, several genera are numerically significant. Obligate anaerobic types include eubacteria and bifidobacteria, such *as Bifidobacterium bifidum* and *Bifidobacterium infantis* which are isolated from the faeces of breast-fed infants. The genus *Lactobacillus* contains many species that occur in the gut of most warm-blooded animals. Although numerically important in the alimentary tract, their ecological significance has not been conclusively determined.

Table III — Bacteria, their substrates and products in the human large intestine.

Bacteria	**Descr-iption**	**Number** log_{10}/g dry wt faeces mean	range	**Substrate**	**Fermen-tation products**
Bacteroides	G- rods	11.3	9.2-13.5	Saccharolytic	A, P, S
Eubacteria	G+ rods	10.7	5.0-13.3	Saccharolytic, some amino acid fermenting species	A, B, L
Bifidobacteria	G+ rods	10.2	4.9-13.4	Saccharolytic	A, L, f, e
Clostridia	G+ rods	9.8	3.3-13.1	Saccharolytic and amino acid fermenting species	A, P, B, L, e
Lactobacilli	G+ rods	9.6	3.6-12.5	Saccharolytic	L
Ruminococci	G+ cocci	10.2	4.6-12.8	Saccharolytic	A
Peptostreptococci	G+ cocci	10.1	3.8-12.6	As for the clostridia	A, L
Peptococci	G+ cocci	10.0	5.1-12.9	Amino acid fermenters	A, B, L
Methanobrevi-bacter	G+ cocco bacilli	8.8	7.0-10.5	Chemolithotrophic	CH_4
Desulphovibrios	G- rods	8.4	5.2-10.9	Various	A
Propionibacteria	G+ rods	9.4	4.3-12.0	Saccharolytic, lactate fermenting	A, P
Actinomyces	G+ rods	9.2	5.7-11.1	Saccharolytic	A, L, S
Streptococci	G+ cocci	8.9	3.9-12.9	Carbohydrate and amino acid fermenting	L, A
Fusobacteria	G- rods	8.4	5.1-11.0	Amino acid fermentation carbohydrate also assimilated	B, A, L
Escherichia	G- rods	8.6	3.9-12.3	As for streptococci	Mixed acids

A = acetate; P = propionate; B = butyrate; L = lactate; f = formate; e = ethanol; S = succinate.

From MACFARLANE *et al.* 1995 in WHITEHEAD R. *Gastrointestinal and Oesophageal Pathology*, 2d ed., Churchill Livingstone [20]

Several types of spore-forming rods and cocci are also normal inhabitants of the gut. The genus *Clostridium* is probably the most ubiquitous: *C. perfringens, C. bifermentans* and *C. tetani* are regularly isolated, albeit in relatively low numbers, and are of significance in human and veterinary medicine. The presence of aerobic members of the genus *Bacillus* is thought to result from contamination from the environment. Facultative and obligately anaerobic Gram-positive cocci are also numerically important. The strict anaerobes include *Peptostreptococcus, Ruminoccus, Megasphaera elsdenii* and *Sarcina ventriculi*. The facultatively anaerobic streptococci are well represented by many species from LANCEFIELD group D including *S. faecalis, S. bovis* and *S. equinus* and some from group K such as *S. salivarius* which is usually associated with the mouth. Gram-negative anaerobic cocci include *Veillonella* and *Acidaminococcus*.

Although they are not numerous, the Gram-negative facultative anaerobic rods include a number of very important pathogens. Members of the Enterobacteriaceae, particularly *Escherichia coli*, are usually thought of as characteristic intestinal bacteria. Several types of spirochaete can be seen in the gut of healthy animals, but their status in the human colon is uncertain.

2-3 Function

Some broad idea of the function of the large intestine can be deduced from a comparison of the amounts and composition of material entering from the ileum and the composition leaving as faeces.

Figure 3a [21] shows the composition of ileostomy effluent and **figure 3b** [22] the composition of faeces from two separate experiments. The control diets in both cases were typical UK diets. From these data it is evident that the dry matter of faeces is about 25 g a day whilst the dry matter of ileal effluent approaches 60 g a day in subjects on similar diets. Ileostomy effluent contains about 16 g of carbohydrate and about 14 g of protein with small amounts of fat and minerals. Examination of faeces, however, shows that the major component is biomass—representing about 15 g per day—with small amounts of carbohydrate, most of which is residual non-starch polysaccharide (NSP) and some water-soluble material, principally organic anion and minerals. About 60 g of dry matter, therefore, is entering the colon each day and about 25 g leaving it. Most of the organic material has been fermented and the products either absorbed or converted to biomass.

Subjects on a high starch diet pass between 90 and 100 g of dry matter into the large intestine, of which approximately one half is carbohydrate—in this case, 35 g of starch and about 15 g of NSP. Most of this starch and NSP is fermented in

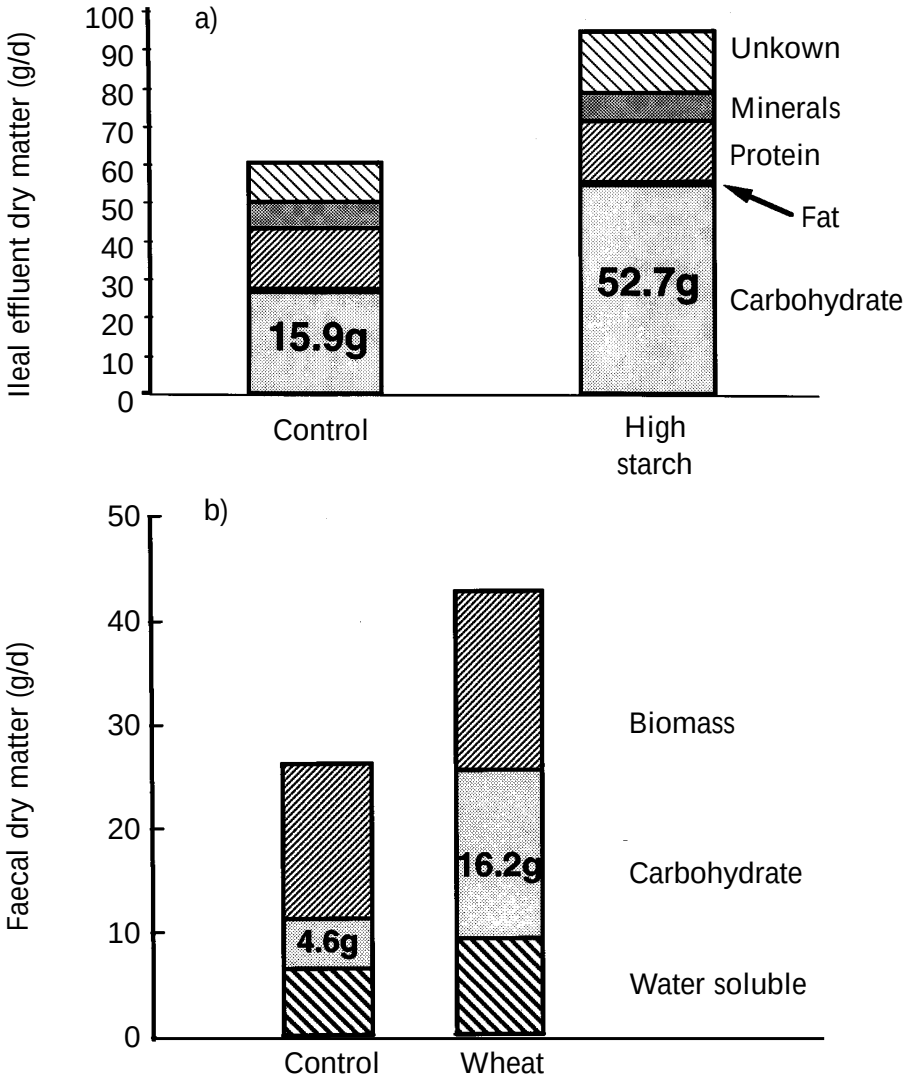


Figure 3 — a) Composition of human ileostomy effluent in subjects taking either a standard UK diet (control) or a high starch diet. The unknown fraction includes some moisture. Mineral, protein and fat excretion are relatively constant, whilst carbohydrate excretion can be seen to change substantially (from SILVESTER *et al.* [21]).

b) Composition of faecal dry matter in healthy subjects eating either a standard UK diet (control) or one to which has been added wholewheat, cereals and bran. In contrast to ileal content, much less carbohydate is excreted and the major component of the dry matter is biomass, resulting from the fermentation of the carbohydrate. The carbohydrate excretion on the high bran diet is atypically high in that this is one of the most poorly fermentable sources of carbohydrate (from STEPHEN and CUMMINGS [22]).

the colon (see Chapter 3). Subjects eating a high NSP diet with most of the NSP coming from wheat excrete a significant amount of NSP in faeces, together with increased biomass and some increased mineral and organic anion excretion. In these subjects biomass is the dominant component of faeces.

A more traditional view of the role of the colon can be obtained by comparing the electrolyte composition of ileal effluent with that of faeces (**Figure 4**). Most of this work was done in the 1970s when the principal role of the colon

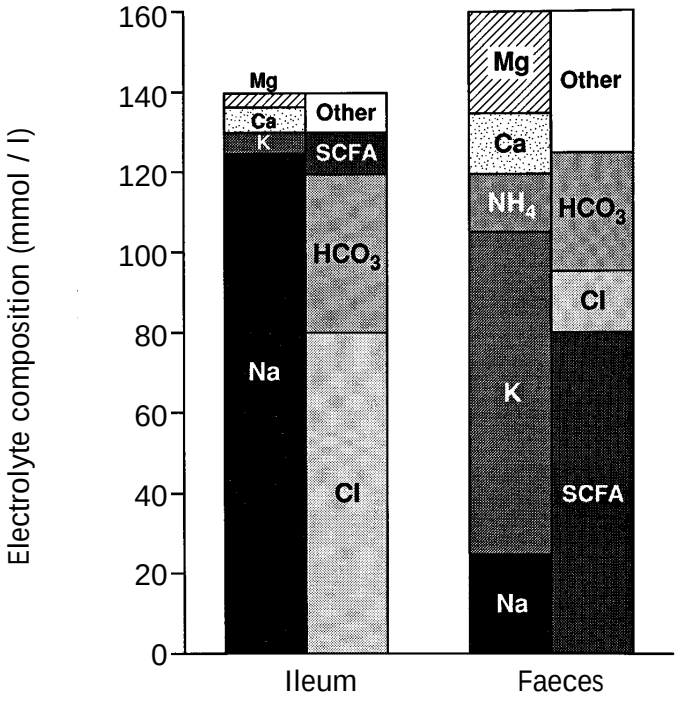


Figure 4 — Electrolyte composition of the free water in human ileal contents and faeces. The osmolarity of ileal contents is about 300 mosmol per kg, whilst that of faeces is more like 380 mosmol per kg. Both cation and anion composition contrast markedly. Ileal contents are principally a saline-bicarbonate solution, whilst faeces are a more complex mixture with short-chain fatty acids occupying a more prominent role and the cation composition being more characteristic of that found intracellularly (compiled from [10,23,24,25]).

was thought to be water and electrolyte absorption. The electrolyte composition of ileal effluent is principally that of a saline solution with some potassium bicarbonate present. Only low concentrations of other ions can be identified and the overall pH is about 6.5. Faecal composition is much more difficult to measure because the free water phase of faeces probably doesn't exist in real life. The ions in such extra-cellular water as exists comprise principally SCFA with small

amounts of bicarbonate, chloride and traces of sulphate and phosphate. The major cations are potassium, calcium and magnesium with some sodium. When the volumes of ileal water (probably over 1l a day) and faecal water (probably no more than 100 ml a day) are calculated, then it can be seen quite clearly that the colon is absorbing substantial amounts of water, sodium and chloride and generating short-chain fatty acids.

All this evidence, together with the bacteriology, points to a very clear function for the colon, namely the breakdown of carbohydrate and protein, by bacteria through fermentation to organic anions, most of which are absorbed. In the course of this process, bacterial growth (biomass) is stimulated and a variety of other end products are formed. The principal metabolic pathways in carbohydrate and protein fermentation are summarised in **figures 5a** and **5b**.

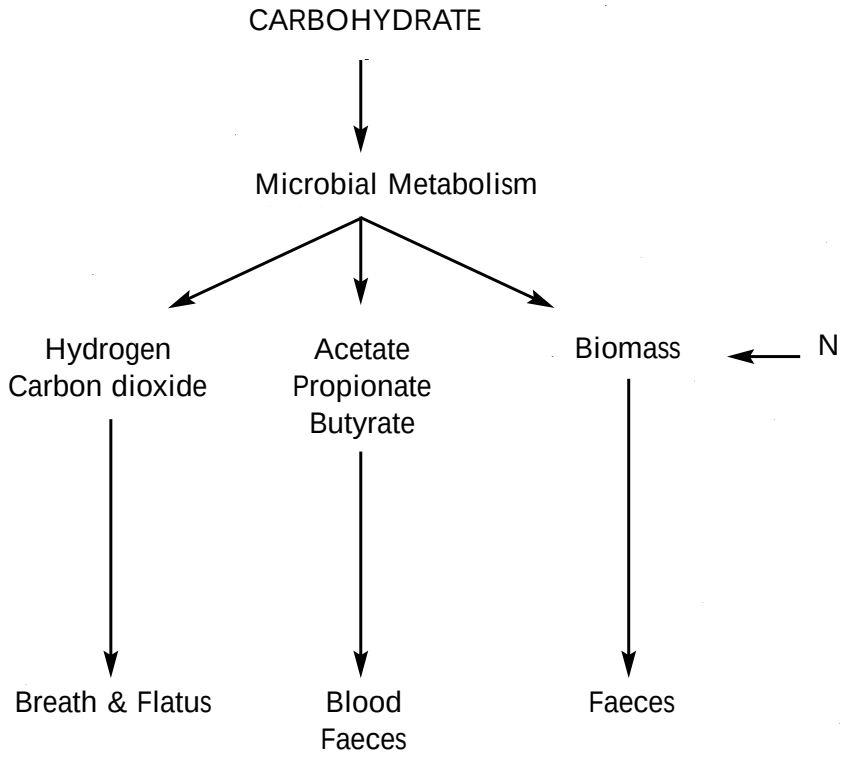


Figure 5a — Overview of carbohydrate fermentation in the human large intestine.

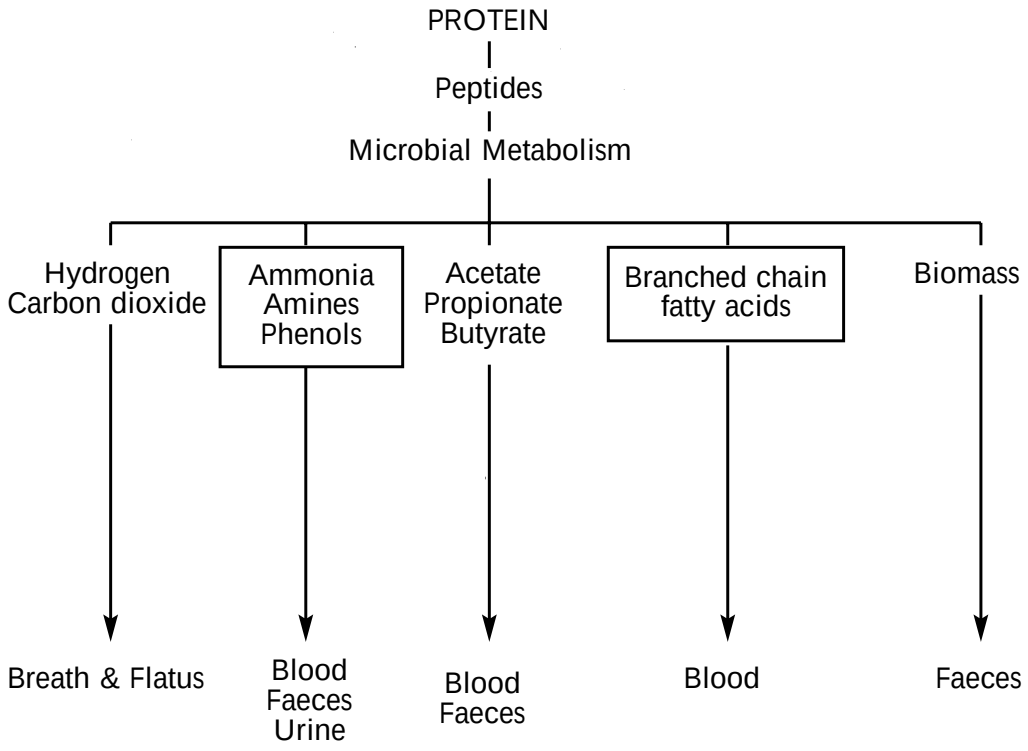


Figure 5b — Overview of protein breakdown and amino acid fermentation in the human large intestine. The products outlined by the boxes are unique to protein fermentation.

These fermentative processes are the key events in the large intestine governing all aspects of its function.

2-3-1 Regional Differences

It has already been noted (§ 2-1) that there are differences between the caecum and sigmoid colon in the concentrations of SCFA, moisture and pH. The reason for this lies in the fact that the caecum and right side of the colon is relatively rich in carbohydrate, and active fermentation at this site yields principally SCFA. The sigmoid region is more carbohydrate depleted, and protein breakdown and amino acid fermentation become more dominant. Here branched-chain fatty acids accumulate along with phenols and amines, and the characteristics of the bacterial flora change towards a more methanogenic and sulphate-reducing type of flora. These contrasts are summarised in **figure 6**.

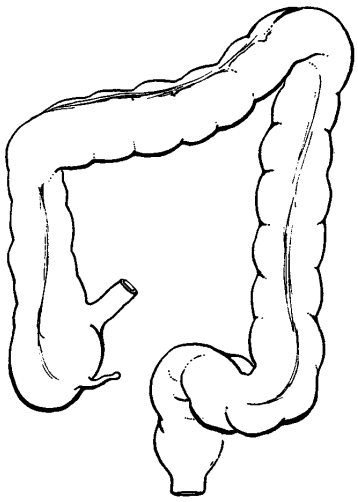
Right side		Left side
Carbohydrate rich		Protein rich
Moisture +		Less free water
SCFA +		SCFA less
pH acid		pH near neutral
Residence time 6–16 h		Residence time 12–36 h
Bacterial growth +		Bacterial growth slower
Mainly H_2 and CO_2		H_2, CO_2 and CH_4
		Amines, phenols and ammonia

Figure 6 — Regional differences in large bowel function in the human.

Even in the human, therefore, the large intestine is a fermentative organ with substantial potential for water, electrolyte and organic anion absorption. The marked regional differences which occur may go some way to accounting for the regional differences in disease activity in different parts of the colon. For example, ulcerative colitis commences in the rectum and spreads proximally, whilst diverticular disease is principally a disorder of the muscle of the sigmoid colon. About 60% of large bowel cancers lie within the rectum, sigmoid and descending colon, whilst a number of the disorders leading to constipation—particularly recto-anal ones—are clearly disorders in the left side of the colon.

3 – Carbohydrate and Protein Digestion: the Substrates Available for Fermentation

3-1 Introduction

Fermentation is driven very largely by the amount and type of substrate available to the bacteria. Unlike the ruminant animal, however, where the whole diet enters the fermentation chamber, the hindgut has to rely on what is not digested and absorbed higher up in the gut. The function of the large bowel is thus closely tied to digestive physiology in the small intestine, and an understanding of the role of the large bowel requires knowledge of digestive events in the upper gut.

The major substrates for fermentation are fairly well known and are shown in **table IV**. The numbers are based on people eating Western style diets and will be considerably different in those living on starchy staples in non-industrialised communities in Africa and India.

3-2 Carbohydrates

Our understanding of carbohydrate digestive physiology has changed substantially in recent years. The polysaccharides of the plant cell wall (fibre) are no longer thought of as inert material passing through the gut and simply affecting bowel habit. They consist of a range of polymers with contrasting physiological effects. Starch is known now not to be rapidly and completely digested in the small bowel and the concept of resistant starch has been developed [29–32] with its attendant implications for glycemic control and large bowel function. More recently the importance of a previously neglected group of carbohydrates, the oligosaccharides, has emerged with the demonstration that they selectively affect microbial function in the large intestine [33]. With this knowledge has come a call

for better food labelling of dietary carbohydrate and therefore a new look at its classification and measurement.

Table IV — Substrates available for fermentation in the human colon.

Type		**Amount** (g/day)
Carbohydrate	Resistant starch	5 – 35
	Non–starch polysaccharides	10 – 25
	Oligosaccharides	2 – 8
	Sugars and sugar alcohols	2 – 5
	Synthetic carbohydrate, *e.g.* lactulose, polydextrose, pyrodextrins, modified celluloses	Unknown
Protein	Dietary (N x 6.25)	1 – 12
	Endogenous, *e.g.* pancreatic enzymes and other secretions	4 – 8
	Urea, nitrate	0.5
Other	Mucus	? 3–5
	Bacterial recycling	Unknown
	Sloughed epithelial cells	Unknown
	Organic acids	Unknown
	TOTAL Carbohydrate	20 – 60
	Protein	5 – 20

From CUMMINGS and ENGLYST [27], CUMMINGS *et al.* [28] and unpublished data.

3-2-1 Classification of Dietary Carbohydrates

In order to understand carbohydrate digestion and physiology it is necessary to have some sort of systematic classification for the many food components which come under this heading.

A classification of food carbohydrate for nutritional purposes must fulfil many criteria; namely, it should:

– include all known dietary substances that are defined by the term “carbohydrate”;
– allow clear boundaries to be drawn between groups;
– provide the analyst with an achievable objective;
– give the consumer and other users useful and reliable information.

Carbohydrates are polyhydroxyaldehydes and ketones that have the empirical formula $(CH_2O)_n$. For nutritional purposes they are best classified primarily according to the degree of polymerisation with subdivisions based on glycosidic linkages and chemistry of individual sugars because these determine physiological properties. **Table V** shows such a classification, which provides a logical approach to food carbohydrate and defines categories which are readily measurable analytically. Although it is possible to determine in the laboratory all the carbohydrate fractions listed in **table V** with reasonable accuracy, no method exists which will divide them physiologically into, for example, digestible and undigestible (available and unavailable) fractions. The physiological properties in the gut of the different chemical categories of carbohydrate overlap in many respects.

The boundaries between the three main divisions—sugars, oligosaccharides and polysaccharides—are reasonably well defined by convention but present some analytical and labelling problems. This is particularly so for the boundary between oligosaccharides and polysaccharides because it is conventionally defined as separating carbohydrates with about 10 or more monomeric units. In analytical terms, this cut-off is provided by polysaccharides which are precipitated in 80% ethanol. However, some carbohydrates straddle both the analytical and labelling boundary. For example, both polydextrose, pyrodextrins and many fructans contain a variety of carbohydrate fractions which occur in both the oligosaccharide and polysaccharide categories. Inulin—a fructan which, in physiological terms, belongs to the oligosaccharides—spans this group with a degree of polymerisation (DP) of anything from 6 to 100. A better term to encompass all these carbohydrates would probably be short-chain carbohydrates but then one would still be left with the problem of deciding an upper limit.

3-2-2 Sugars

Sugars add sweetness to food and make it pleasant to eat, hence the use of table sugar—which is sucrose, a disaccharide of glucose and fructose—to sweeten drinks, breakfast cereals, fruit desserts, tomato ketchup, etc. High concentrations of sugar aid in the preservation of jams and pie fillings, and sugars are important in creating the texture and structure of cakes, biscuits and confectionery. Most sugars are absorbed from the small intestine and, through the maintenance of blood glucose, provide rapidly available energy to the tissues. Sugars are not normally a substrate for fermentation. Sucrose, and occasionally other sugars, may be malabsorbed but only in pathological conditions such as jejuno-ileal bypass. Dietary lactose from milk does pass into the large intestine in people with β-galactosidase deficiency and is fermented since this enzyme is widely present in bacteria. Lactose is rarely found in adult human faeces. The rise in consumption

Table V — Classification of dietary carbohydrates.

Group	**DP** (1)	**Sub-group**		**Digestion in the small intestine**
Sugars	1	Monosaccharides	Glucose, fructose, galactose, sorbitol, mannitol	Usually well absorbed except sugar alcohols
	2	Disaccharides	Sucrose, lactose, maltose	Usually well absorbed except lactose
Oligosaccharides	3–9	α-glucans	Mostly starch hydrolysis products	Well digested
		Non α-glucans (NDO)	Fructo-oligosaccharides, galacto-oligosaccharides, raffinose, stachyose, polydextrose (partly)	Probably all reach the caecum
Polysaccharides	≥10	Starch (α-glucans)	Amylose, amylopectin	Some forms of resistant starches reach the caecum
		Non-starch polysaccharides (non α-glucans)	Cell wall Cellulose Hemicellulose Pectins, etc.	All reach the caecum
			Storage Guar Inulin	All reach the caecum
			Gums, exudates, mucilages Ispaghula Sterculia Karaya	All reach the caecum

(1) Degree of polymerisation.

Modified from ENGLYST *et al.* [30].

of sucrose from 1850 to 1950 has led to the claim that it is responsible for dental caries and may contribute to obesity, diabetes and cardiovascular disease. However dental caries prevalence has declined dramatically over the past 30 years, probably as a result of fluoridation of drinking water and toothpaste. Current epidemiological evidence does not support a role for sugars in the cause of cardiovascular disease or diabetes or obesity [34–36].

3-2-3 Oligosaccharides

Oligosaccharides are carbohydrates with a degree of polymerisation (DP) of 3–10 [37]. They are readily soluble in water and include tri- and tetrasaccharides such as raffinose and stachyose, maltodextrins from partial hydrolysis of starch, pyrodextrins produced by heating starch in acid conditions, fructo- and galactooligosaccharides, and some inulins. Pyrodextrin preparations are marketed in Japan for their health-giving properties as drinks and are thought to be poorly digested. The trisaccharide raffinose (Gal-Glc-Fuc) and tetrasaccharide stachyose (Gal-Gal-Glc-Fuc) are found in legumes and, because of their non-digestibility in the small bowel, provide substrates for bacterial fermentation in the colon, especially gas formation [38, 39]. Several other possible health benefits are currently of interest [33, 40].

It is, however, the fructooligosaccharides that have aroused interest in recent years, mostly because of their property to stimulate the growth of bifidobacteria in the colon, first described by YAZAWA and colleagues [41, 42]. Polymers and oligosaccharides of fructose are synthesised by many plants and are found in the human diet in onions, garlic, artichokes and chicory, which provide up to 12 g/d [43, 44]. The best known are the inulins, a group of β-(2-1) linear fructose polymers with a DP ≤ 60. Fructooligosaccharides are produced in two ways, either by partial hydrolysis using endoglycosidases, for example Raftilose (Orafti, Tienen, Belgium), which is made from chicory inulin, or by synthesis from sucrose by using fungal β-fructofuronosidase, *e.g.* Neosugar (Meiji, Kanagawa, Japan) [45].

Fructooligosaccharides may be partly hydrolysed by gastric acid [46, 47], but in general they are thought to escape digestion in the human upper intestine completely [33, 43, 48]. Inulin, a closely related carbohydrate, has been shown in a recent study [49] to largely (87%) escape digestion in the small bowel. Oligosaccharides are fermented *in vivo* by the colonic microflora [50-52]. Their most important property, which may be unique, is to stimulate bifidobacterial growth specifically while suppressing the growth of some other species such as *Clostridium perfringens* (**Figures 7a** and **7b**).

In vitro fructooligosaccharides are fermented mostly to acetate and propionate with some butyrate and lactate, H_2, and CO_2. In batch cultures both oligofructose and inulin specifically stimulate bifidobacterial growth [42, 53, 54],

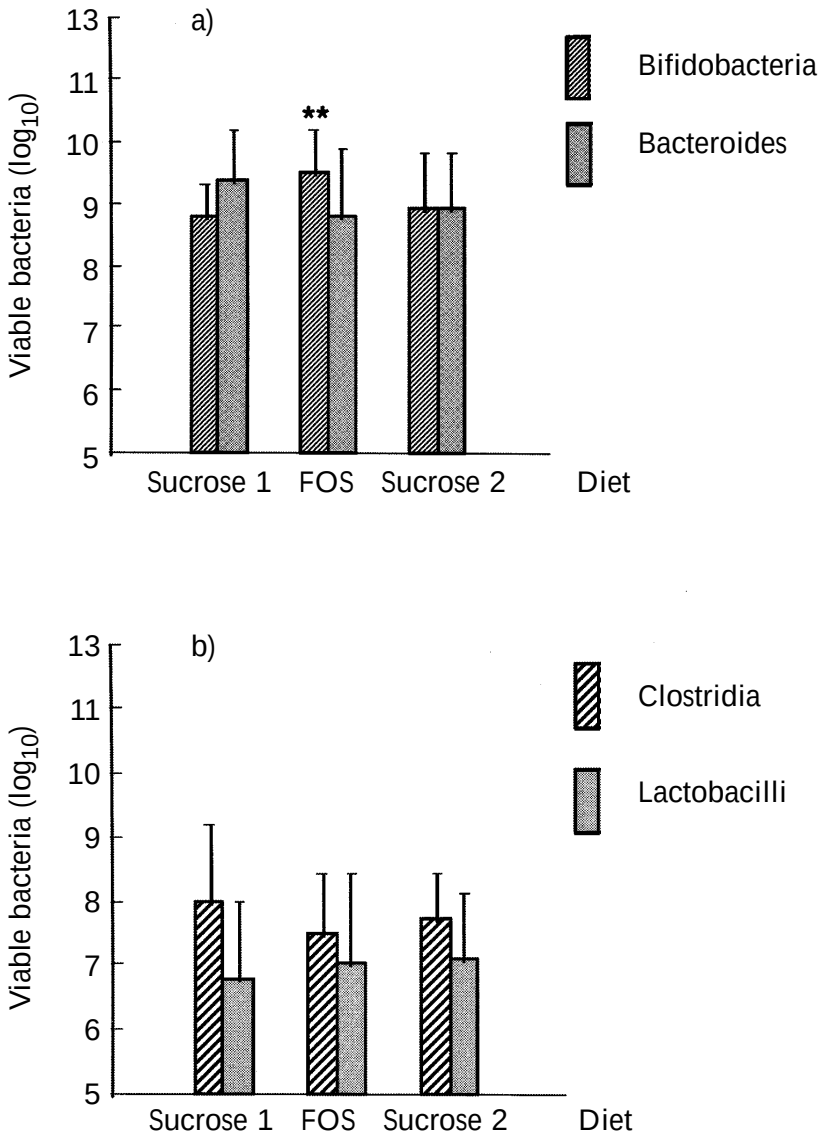


Figure 7 — Effect of fructooligosaccharides (FOS) on viable counts of :

a) bifidobacteria and bacteroides in eight healthy subjects during 15 day dietary feeding periods. FOS significantly increased bifidobacteria and decreased bacteroides numbers such that bifidobacteria became the dominant organisms in these subjects' faeces;

b) clostridia and lactobacilli in the same study. FOS significantly reduced clostridia (and fusobacteria) during the feeding period (from GIBSON *et al.* [52]).

an effect that has been confirmed in both rats and humans [42, 52, 55]. In contrast, oligofructose, inulin and starch inhibit the growth of clostridia. The mechanism for this inhibition has been thought to be related to lowering of intestinal pH by bifidobacterial fermentation. However, GIBSON and WANG [56] showed that factors other than pH lead to repression of the growth of clostridial species. Bifidobacteria are generally regarded as being of benefit to health, hence their addition to yoghurts in many countries. The health benefit is allegedly related to stimulation of immune function, vitamin B synthesis, restoration of a normal flora after antibiotic therapy, and prevention of growth of pathogenic species. They are the major flora in breast-fed infants and this is one mechanism whereby these children are protected against gut infections. Fructooligosaccharides may also affect lipid metabolism, decreasing triglyceride and cholesterol concentrations in rats [33, 57, 58] and in diabetic humans [59]. **Table VI** summarises the health benefits of oligosaccharides.

Table VI — Potential health benefits of oligosaccharides.

Substrates for fermentation
Short-chain fatty acid production
Biomass (laxative effect)
Reduced nitrogenous end products in colon (ammonia, amines)
Selective stimulation of bifidobacteria
Protection against invading pathogens
Suppression of growth of clostridia and coliforms
Lipid metabolism
Decrease in triglyceride synthesis
(No effect on cholesterol)
Stimulate immune function

3-2-4 Starches

Starches are the major storage polysaccharide in most higher plants. They are α-glucans and exist in two forms: amylopectin, which is 70–80% of total starch, and amylose, which usually comprises 20–30%. Amylopectins are large molecules made up of >10,000 glucose residues held together by α-1,4 and α-1,6 linkages in a branched tree-like structure. Amylose is a smaller linear molecule with only α-1,4 linkages. Starch is stored in plants as partially crystalline granules,

which in amylopectin have clusters of interchain branching points and domains of short chains. These short chains exist as double helixes and give rise to an alternating pattern of crystalline (arrays of double-stranded helixes) and amorphous (zones of dense branching) regions [60]. This partially crystalline structure has distinct patterns on x-ray diffraction. Three main types have been described: A, B and C. Type A is thermodynamically the most stable form and is found in cereal starches. The B form is characteristic of banana, potato and other tubers and the C pattern is found in legumes. The size and crystalline nature of starch granules influence their susceptibility to pancreatic enzymes. In general, starch granules showing x-ray diffraction patterns B and C tend to be more resistant to pancreatic amylase.

Starches are insoluble in cold water but on heating they swell and eventually their molecular organisation breaks down and loses its crystallinity (**Figure 8**). The granule is totally disrupted and at this stage starch is readily

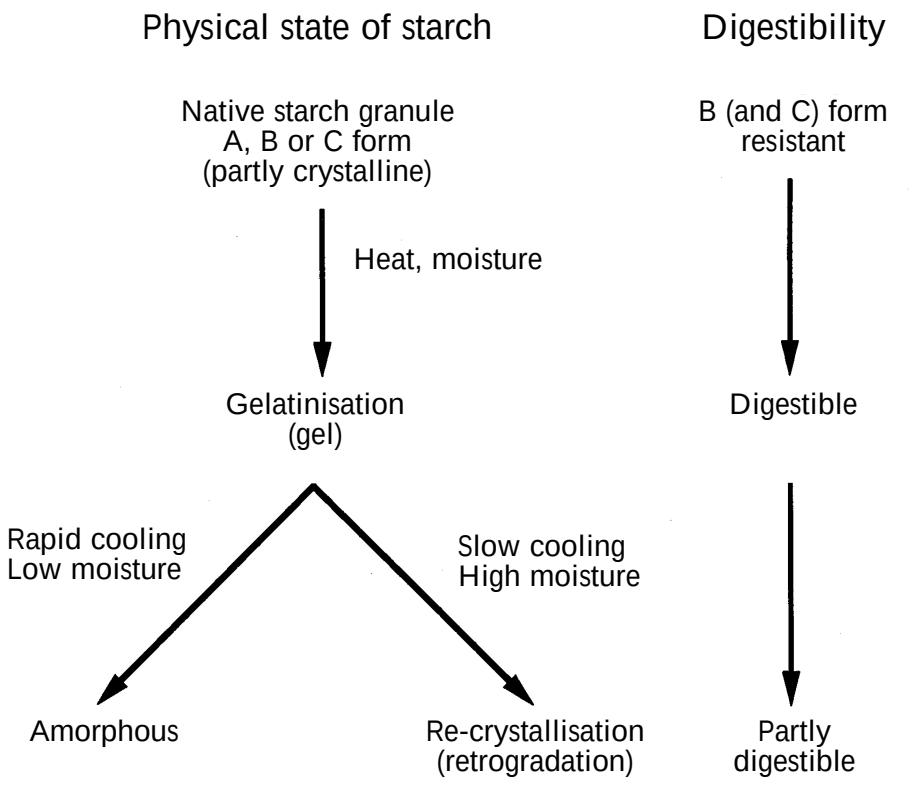


Figure 8 — Physical state of starch in relation to its digestibility. In its native form, some starches are resistant to digestion but on gelatinisation all starches become susceptible to pancreatic amylase. Cooling, however, can lead to re-crystallisation and a decrease in digestibility (modified from [60] and [61]).

hydrolyzed by enzymes. The whole process of disruption and swelling is known as gelatinisation [60] and is the essence of cooking starchy foods. On cooling, gelatinised starch recrystallises, a change known as retrogradation. Retrograded starch, particularly amylose, is more resistant to enzymic hydrolysis. The physical determinants of starch gelatinisation and recrystallisation are complex but are of vital importance for starch digestion because even simple food processes such as heating and cooling which occur during the preparation of a meal will affect the nutritional quality of starchy foods.

Starch digestion

In the past decade two widely held assumptions concerning the digestion of starch have been disproved. The first is that all starch, because it exists as large complex polymers, is hydrolysed and absorbed more slowly than are simple sugars or disaccharides. Several studies *in vitro* and *in vivo* have shown that the physical form of food is the major determinant of the rate of digestion of both starches and sugars [62–65]. The second wrong assumption is that starch is completely hydrolysed and absorbed within the small intestine. It is now known that the extent of starch digestion within the small intestine is variable and that a substantial amount of starch, depending again on physical form, escapes digestion in the small intestine and enters the colon [30]. The reasons for the incomplete digestion of starch may be separated into intrinsic factors (*i.e.* properties of the food) and extrinsic factors (*e.g.* chewing and transit through the bowel).

– Intrinsic factors

Starch digestion is slowed in the small intestine if the physical form of the food hinders access of pancreatic amylase. This occurs if starch is contained within whole or partly disrupted plant structures such as grains or seeds; if rigid cell walls inhibit swelling and dispersion of starch, as in legumes [64, 66] or if starch is very densely packed in a food such as spaghetti [67]. When the rate of starch digestion is decreased, postprandial glucose and insulin responses are reduced or delayed. HEATON *et al.* [68] demonstrated that these responses are smaller if wheat, maize or oats are given as whole or coarsely ground grains than if given when finely milled. When hydrolysis is grossly retarded, physically inaccessible starch may enter the colon and be fermented or, in some cases, appear in faeces [69].

Starch granules which show x-ray diffraction patterns B and C (*e.g.* in potato, banana and legumes) are more resistant to pancreatic amylase than are those of pattern A (in cereals). Ungelatinised granules of the B form may survive cooking such as the baking of biscuits. When starch granules are fully gelatinised and dispersed, the starch becomes easily digestible. However, as the gel cools and

ages, the polymers once more form a partially crystalline structure. Recrystallisation or retrogradation depends on the formation of interchain hydrogen bonds and occurs most rapidly for the linear amylose. Retrogradation of amylopectin is limited by its branched structure and the polymers of retrograded amylose. Retrograded starch characteristically forms the B-type pattern.

Other factors intrinsic to starchy foods that have been shown to affect α-amylase activity *in vitro* include amylose-lipid complexes [70], native α-amylase inhibitors [71] and NSPs which may have a direct effect on enzyme activity [72].

– Extrinsic factors

Although the effect of intrinsic factors on digestion of starchy foods may be measured in a reproducible way, this does not necessarily predict exactly the rate and extent of digestion of these foods by individuals. This is because eating exposes food to variable external influences which may alter the susceptibility of the starch to hydrolysis by pancreatic amylase. For example, the extent of chewing determines the physical accessibility of starch contained within rigid structures [73]. Other factors are the transit time of the food from mouth to terminal ileum [74], the concentration of amylase in the gut, the amount of starch present and the presence of other food components that might retard enzymic hydrolysis.

Classification and measurement of starches

A classification of starch for nutritional purposes which is based on the intrinsic factors affecting starch digestibility is shown in **table VII**. In view of the importance to health of starch there is a need to have good laboratory methods for its measurement for both research purposes and for food tables and labelling. A single value for total starch is of limited use because of the widely varying digestibility of different starch types.

The various categories of starch shown in **table VII** may be determined in the laboratory by controlled enzymatic hydrolysis and measurement of the released glucose as described by ENGLYST. Total starch (TS) is measured as the glucose released by complete enzymatic hydrolysis of starch after gelatinisation in boiling water and treatment with potassium hydroxide to disperse retrograded amylose. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) are measured after incubation with pancreatic amylase and amyloglucosidase at 37ºC. A value for rapidly digestible starch is obtained as the glucose released after 20 min and slowly digestible starch as the glucose released after a further 100 min incubation. Resistant starch (RS) is calculated as the starch not hydrolysed after 120 min incubation. If required, separate values may be obtained for physically inaccessible starch (RS1), resistant starch granules (RS2) and retrograded amylose (RS3) [30].

Table VII — Classification of starch for nutritional purposes.

Type of starch	**Example**	**Probable digestion in small intestine**
Rapidly digestible starch (RDS)	Freshly cooked starchy food	Rapid
Slowly digestible starch (SDS)	Most raw cereals	Slow but complete
Resistant starch (RS)		
Physically inaccessible starch (RS1)	Partly milled grains and seeds	Resistant
Resistant starch granule (RS2)	Raw potato and banana	Resistant
Retrograded starch (RS3)	Cooled, cooked potato, bread and cornflakes	Resistant

From ENGLYST *et al.*, 1992 [30].

Table VIII shows values for rapidly digestible starch, slowly digestible starch, resistant starch and total starch expressed as % dry matter for foods. The highest amount of rapidly digestible starch (73–74%) was found in Cornflakes and Rice Krispies. Breads and potato also contained substantial amounts (56–71%) of rapidly digestible starch. The lowest values (3–8%) were found for banana flour, raw potato starch, and haricot beans. The amount of slowly digestible starch is inversely related to the amount of rapidly digestible starch and was therefore low for breads and breakfast cereals and relatively high for foods such as spaghetti and legumes in which the physical form of the food inhibits access of pancreatic amylase to the starch.

The products containing the most resistant starch were raw potato starch and banana flour. The large amounts of resistant starch in raw potato starch and banana flour reflect their content of starch granules with the B-type crystal structure, which is highly resistant to pancreatic amylase. Potato biscuits made from 50% wheat flour and 50% potato starch also contained a substantial amount of RS2, including incomplete gelatinisation of starch granules during baking. The small amounts of resistant starch in breads and breakfast cereals are mainly retrograded amylose (RS3). The resistant starch found in cold potato, spaghetti and legumes is partly RS3 and partly physically inaccessible starch, RS1. Of the

Table VIII — Digestibility *in vitro* of some carbohydrate-containing foods.

Sample	Dry matter (%)	Types of starch (g/100 g dry matter)				RAG (g/100 g wet wt)
		RDS	SDS	RS	TS	
White bread	54.5	69	7	1	77	42
Wholemeal bread	52.0	56	4	1	60	32
Ready Brek	92.4	62	3	1	66	64
Shredded Wheat	91.3	66	4	-	71	68
Rice Krispies	95.6	74	2	1	78	84
Cornflakes	95.8	73	2	3	78	81
Porridge oats	90.7	57	6	2	65	58
Digestive biscuit	94.2	37	10	1	48	47
Rich tea biscuit	96.0	42	9	1	52	54
Lincoln biscuit	95.6	23	23	-	46	35
Abbey Crunch biscuit	96.1	22	13	1	35	41
Ryvita crispbread	94.3	52	6	3	61	56
Potato biscuit	94.9	23	17	15	55	30
Instant potato	16.7	72	1	1	73	13
Boiled potato (hot)	22.8	65	5	5	74	16
Boiled potato (cold)	23.8	53	11	10	75	14
Potato starch (raw)(1)	81.8	6	19	75	99	5
Banana flour (1)	99.1	3	15	57	75	6
High-amylose maize starch (1)	95.2	70	11	17	98	74
Maize RS product (1)	92.5	55	9	34	98	57
White wheat flour	89.7	40	39	2	81	40
Spaghetti (freshly cooked)	28.3	41	33	5	79	13
Spaghetti (cooled)	34.7	33	42	4	78	13
Pearl barley (boiled 1h, cold)	23.3	34	30	9	73	9
Millet (boiled 20 min, cold)	27.1	42	28	6	75	13
Peas (frozen, boiled 5 min)	18.3	12	2	5	20	5
Lentils (boiled 20 min, cold)	28.3	24	22	9	54	8
Haricot beans (boiled 40 min)	41.4	8	19	18	45	4
Bean flakes (1)	93.6	27	16	6	49	29
Soluble starch	100	100	-	-	100	110
Glucose	100	-	-	-	-	100
White cabbage (raw)	8.9	-	-	-	-	3
Red pepper (raw)	7.0	-	-	-	-	2
Apple (Granny Smith, raw)	11.8	-	-	-	-	3

RDS = rapidly digestible starch; SDS = slowly digestible starch; RS = resistant starch; TS = total starch; RAG = rapidly available glucose.

(1) Samples from the EURESTA trial, described in text.

From ENGLYST *et al.* [30].

common foods analysed, haricot beans (soaked overnight and boiled for 40 min) contained the most resistant starch, which constituted 40% of the total starch and comprised all three resistant starch fractions.

During the measurement of rapidly and slowly digestible starch, a value is obtained for rapidly available glucose (RAG) as the glucose measured after 20 min incubation with pancreatin, amyloglucosidase, and invertase (G_{20}) :

RAG = free glucose + glucose from sucrose +

+ glucose released from starch within 20 min incubation.

The rapidly available glucose value thus represents the amount of glucose (in % by wt) that can be expected to be rapidly available for absorption after a meal. Rapidly available glucose, expressed as a percentage of total starch plus free sugars, shows a correlation with the glycemic index [62]. The foods in **table VIII** show a wide range in their content of rapidly available glucose. For example, the rapidly available glucose in a 35 g-portion of cornflakes (28 g glucose) corresponds with that in 68 g white bread, 88 g wholemeal bread, 175 g freshly cooked potato and 700 g haricot beans (cooked as described).

Starch digestion in vivo

The amount of starch which escapes digestion in the human small bowel each day is difficult to quantify. **Table IX** shows results from a series of ileostomy studies in humans. From the table it will be seen that cereal starches are on the whole well digested, with only 2 to 3% escaping in ileostomy effluent, unless the starch has been processed and retrogradation has taken place (RS3). The same is true for freshly cooked potato, but once cooled 12% becomes amylase resistant. Banana starch escapes digestion almost entirely. In another series of ileostomy studies, CHAPMAN *et al.* [74] found only 2.4% (1.9 g) of starch malabsorbed from meals of wheat and potato starch, while WOLEVER *et al.* [79] found total carbohydrate passing to the large intestine after test meals to be 11% (9.5 g) from white bread, 8% (7.7 g) from wholemeal bread and 18% (20.8 g) from red kidney beans. In studies of healthy people intubated so that direct measurement of carbohydrate passing through the ileum could be made, STEPHEN *et al.* [80] found that 6% and 9% (2.1 and 4.8 g) of the carbohydrate from two mixed meals were malabsorbed.

A number of studies of starch digestion have relied on the appearance in breath of hydrogen generated during fermentation as an index of starch breakdown. The amount of hydrogen in breath is compared to that released during fermentation of a known amount of carbohydrate such as lactulose and, from this ratio, quantitative data for starch absorption are derived. For example, LEVITT's group, in a series of studies using breath hydrogen as a marker of carbohydrate

Table IX — Starch and NSP losses from the human small intestine (g per test meal in ileostomy subjects).

Meal	NSP			Starch			
	Fed (g)	Recovered (g)	(%)	Fed (g)	(RS)	Recovered (g)	(%)
White bread	2.3	2.3	100	62		1.6	2
Oats	6.6	6.3	95	58		1.2	2
Cornflakes	0.6	0.7	100	74		3.7	5
Banana	2.1	2.0	95	19		17.2	90
Potato hot	3.3	3.4	100	45		1.5	4
cooled				47			12
Wheat flour				51	(0.3)	0.5	1
Potato flour				51	(11.7)	13.9	27
Banana flour				47	(15.0)	13.9	30
Maize RS3				47	(8.5)	8.6	18
Wheat RS3				47	(8.5)	9.0	19
						Mean	*21*

Data from [75–78].

malabsorption, have calculated that 10 to 20% of the 100 g of carbohydrate in a meal of white bread is not absorbed and neither is 8% in oat bread, 6% in corn bread, 13% in potato and 18% in navy beans [81, 82]. When contrasted with the data from ileostomy studies and direct intubation, the breath hydrogen technique seems to give higher values for starch malabsorption [83]. This is because a number of assumptions are made about fermentation in breath hydrogen studies which may not be universally correct.

Despite the problems in interpretation, it is clear that significant quantities of starch arrive in the colon. In a typical Western diet, the amount is probably about 5–10 g/day but can easily be increased to 30–40 g/day by changing a few foods [21]. In countries were starchy foods are the main part of the diet, *e.g.* South India (rice), Uganda (banana) and Southern Africa (maize), and where food is often eaten cold (which will result in starch resistant to α-amylase), then substantially more may escape digestion in the small bowel, all of which provides substrate for fermentation in the colon.

3-2-5 Non-Starch Polysaccharides – Dietary Fibre

The digestive physiology of NSP (dietary fibre) is simple. In humans, all NSP escapes digestion and arrives in the caecum (**Table IX**). The problems arise with the definition and methods of analysis.

Definition of Dietary Fibre

Even after more than 25 years of research into the chemistry, physiology and health benefits of dietary fibre, we still have to ask the question "What is dietary fibre?". This is not because of ignorance — we now know a lot about fibre — but for other reasons. The original description of dietary fibre by TROWELL (1972) [84] namely "that portion of food which is derived from the cellular walls of plants, which is digested very poorly by human beings" is a nutritional concept and not really a true definition. Subsequent attempts to define fibre in the light of new knowledge have either been equally unsatisfactory or have failed to gain international agreement. Fibre means different things to different people, largely depending on your point of view. For example, the consumer sees it as something associated with particular health benefits, the breakfast cereal manufacturer as a useful marketing ploy, the nutritionist as a characteristic of a healthy diet, the analyst as a group of chemical substances and the botanist as part of the plant. All of these views are legitimate in their own context, but not necessarily compatible with a single definition nor always in the best interests of the consumer.

The present concept of dietary fibre came into being around 1970 as a result of the convergence of evidence from epidemiology, physiology and analytical studies, which related the nature of diet to the prevalance of cardiovascular disease, obesity, diabetes, gallstones and various conditions of the large bowel.

HIGGINSON and OETTLÉ [85] made an important early epidemiological observation when they noted in 1960 that constipation and bowel cancer were rare in Africa whilst being common in the West, and that in Africa "a large amount of roughage is consumed .." and "stools are bulkier and more frequent ...". In 1971 BURKITT [86] suggested a specific hypothesis relating lack of dietary fibre to the cause of bowel cancer and a mechanism whereby fibre, through its capacity to "regulate the speed of transit, bulk and consistency of stool ..." and effect microbial metabolism was able to prevent it.

About this time TROWELL, influenced by his experience in Uganda [87] and by reading CLEAVE's *"The Saccharine Disease"* [88], suggested that a number of other chronic Western diseases like obesity, diabetes, coronary heart disease, gallstones might also be due to consumption of fibre-depleted foods. Concurrently

PAINTER, working in Oxford, proposed that diverticular disease was due to colonic stasis induced by lack of fibre [89]. BURKITT and WALKER then added varicose veins and haemorrhoids to the list of fibre-deficiency disorders [14, 90].

Parallel with these epidemiological observations was the development of knowledge of the physiological properties of fibre in the gut. By 1970 there was already a substantial body of such knowledge, which was summarised in early books and reviews [91–93]. These sources stressed principally the large bowel effects of dietary fibre, including the pioneering work of WILLIAMS and OLMSTED [94, 95] and the early studies of bile acid and cholesterol metabolism summarised by STORY and KRITCHEVSKY [96]. Subsequently a number of other properties of fibre became apparent, most notably those that moderate the absorption of other carbohydrates and so may influence the management of diabetes. Possible effects on satiety and obesity, vitamins and mineral absorption, mucin secretion and epithelial cell turnover in the gut were subsequently discovered [97, 98].

Advances were also occurring during this time in methods for determination of dietary fibre in the laboratory, most notable of which were those of SOUTHGATE [99] and, in animal nutrition, of VAN SOEST [100].

The convergence of these lines of evidence gave birth to the dietary fibre hypothesis and stimulated BURKITT and TROWELL to write a book [101]. BURKITT wrote to TROWELL asking him to define “dietary fibre”. TROWELL thought this would be simple but could not find fibre defined in any textbooks of medicine or nutrition [102]. Some food tables listed values for the crude fibre content of foods. Crude fibre, a value obtained by a gravimetric method used to analyse animal feeds, had been in use for over 150 years. However, VAN SOEST and colleagues had already shown that this method failed to recover the majority of the plant cell wall material [100].

In the UK the Medical Research Council’s *Composition of Foods* [103] contained data on “unavailable carbohydrate”, a term used by MCCANCE and LAWRENCE in 1929 [46] and earlier by ATWATER. In trying to prepare accurate food tables for diabetic diets MCCANCE and LAWRENCE tried to distinguish between “available carbohydrate (which) consists of starch and soluble sugars ... and the ... unavailable, mainly ... hemicellulose and fibre (cellulose)”.

TROWELL, however, wanted a generic term for the undigested plant cell wall that he thought was important to health and so he decided to redefine fibre. The earliest formal description of dietary fibre was therefore by TROWELL, who defined it as “the proportion of food which is derived from the cellular walls of plants which is digested very poorly in human beings” [84]. This was largely an attempt to distinguish it from crude fibre. It was a physiological-botanical description which did not identify the precise nature of dietary fibre nor define exactly the limiting characteristics of substances that should be included or excluded in the

description, apart from the reference to plant cell wall material. Moreover, there was no method specified. Implicit in the definition, however, was the belief that this fraction of the diet had special health-giving properties.

Since 1972 TROWELL, and others, have redefined fibre on a number of occasions. What history, however, points to is a fraction of the diet associated with the plant cell wall, believed to be indigestible in man, affecting principally bowel function but also possibly lipid and carbohydrate metabolism, and valuable in the prevention of large bowel disorders, coronary heart disease and diabetes.

– Distinctive features of dietary fibre

There are three possible areas where we might find a unique defining aspect of dietary fibre, these being: its chemistry, its non-digestibility and its physiological/health properties.

• Chemistry. The plant cell wall is a very diverse structure and varies greatly in its composition. In mature cells, however, it is principally composed of a group of polysaccharides which are unique in the human diet in that they contain no α-glucosidic bonds, *i.e.* they are non-α-glucans or non-starch polysaccharides (NSP). Other components of the plant cell wall are usually no more than 10% [104]. A second distinctive feature of the plant cell wall is that it provides a structure to food, and confers physical properties during digestion. Unlike the cell walls of foods of animal origin, the plant cell wall is much thicker, tougher and more resistant to digestion. The plant cell wall therefore gives us two fairly characteristic features by which we might identify dietary fibre : its polysaccharide composition and its physical properties.

• Non-digestibility. The supposed non-digestibility of dietary fibre was originally thought to be a total lack of digestion in the whole gut, i.e. fibre simply passed through from mouth to anus, bulked up faeces by its water holding and was effectively inert. However, the extensive breakdown in man of the polysaccharides of the plant cell wall (cellulose, pectin and other non-cellulosic polysaccharides) was demonstrated by WILLIAMS and OLMSTED in 1936 [94, 95] and many times since (see summary by CUMMINGS in 1981 [105]). But what about non-digestibility in the small bowel? No-one has yet identified human enzymes capable of hydrolysing plant polysaccharides other than starch and a variety of human studies using breath hydrogen, ileostomy subjects and ileal intubation, and *in vitro* techniques have clearly shown that NSP survive intact to reach the colon. However, this is a feature shared by many carbohydates, *e.g.* resistant starches, most oligosaccharides, sugar alcohols and in many populations, lactose.

• Health benefits. Is there such a thing as a true fibre-deficiency disorder, *i.e.* a disease that is induced only by low intakes of dietary fibre? Perhaps the nearest we can get to this is constipation. Physiologically there is no other dietary component at present which shows such a close relation between bowel habit and intake in the diet. However, all malabsorbed carbohydrates can effect laxation, in particular resistant starches [106-109] and oligosaccharides [52], although fibre may well be the most effective in this context. Other large bowel diseases such as diverticular disease and cancer are candidate fibre-deficiency disorders, but current evidence by no means supports an exclusive role for fibre in these conditions like, for example, deficiency of vitamin C leading to scurvy. The case with regard to coronary heart disease, diabetes and obesity is even less convincing, although dietary fibre may contribute. The health benefits of fibre therefore do not provide a distinct defining characteristic or a disease that can be exclusively associated with it, although constipation comes closest to fulfilling this criterion. However, constipation has many causes and laxation is produced by other carbohydrates. Dietary fibre serves as a marker of a diet rich in plant food, many components of which contribute to health.

– Limiting the definition of dietary fibre

We thus have a good case to avoid the concept of non-digestibility and based on chemical and physiological properties, for limiting the definition of dietary fibre to plant cell wall material. From health studies, no distinctive feature of dietary fibre emerges although faecal bulking comes closest. If we define fibre as plant cell wall material we exclude resistant starch, oligosaccharides and non-digestible sugars. Since the dietary fibre content of foodstuffs—especially breakfast cereals—is thought to sell products, some manufacturers argue for the inclusion of as many constituents of the diet as possible under the label of fibre to give a high “fibre” value. This, however, is a very short-sighted view and against both the historical concept of dietary fibre and the consumers’ interest. A more enlightened approach would be a new classification of dietary carbohydrate and public education to back it up. Combining disimilar substances under the label of dietary fibre is unjustifiable physiologically and analytically.

• Lignin. Lignin is a highly insoluble small polymer, the basic units of which are substituted phenylpropanes. It is not a carbohydrate although it is a component of some plant cell walls. Most human foods are eaten at an unlignified stage, the main exception to this being wholegrain cereals. Interest in lignin is largely a carryover from animal studies where lignin forms a part of ruminant feeds and has been shown to retard plant cell wall digestibility. Its physiological effects and health benefits, which are likely to

be different from those of the cell wall polysaccharides, have never been clearly demonstrated in man. Moreover, there are no methods currently available for measuring true lignin that are suitable for general laboratory use. What is usually measured is "KLASON lignin", which contains artefacts of heat treatment, tanins and other phenolics. Including a value for lignin with the plant cell wall polysaccharides would make dietary fibre measurements more difficult to interpret.

• The analyst. The food analyst is a chemist and needs a clear chemical objective. He or she does not want to be involved in assessments of digestibility nor have to measure chemically very disparate substances either with regard to molecular mass or composition. Asking the analyst to measure the plant cell wall is unrealistic but a suitable surrogate exists in the measurement of the non-starch polysaccharides, and for reasons already given these are the most important fraction to measure. Accurate methods which have been tested in international studies exist for NSP. For the analyst, therefore, the measurement of the non-starch polysaccharides of the plant cell wall proves an accurate and attainable objective.

Dietary fibre should therefore be defined as plant cell wall material and measured as non-starch polysaccharides.

Analysis of Dietary Fibre

There are two principal methods of analysis for dietary fibre both of which have undergone extensive development and interlaboratory testing over a period of ten years. These two methods are characterised by being either enzymatic gravimetric or enzymatic chemical.

– The enzymatic gravimetric methods [110–114]

The principal method, adopted by the Association of Official Analytical Chemists (AOAC), was described first in 1984 by PROSKY *et al.* [110]. The aim of the method was that it should be rapid and measure indigestible carbohydrate as "the sum of the soluble and insoluble polysaccharides and lignin". In principle it involves gelatinising dried foods with a heat stable α-amylase and then enzymatically digesting them with protease and amyloglucosidase to remove protein and starch. Ethanol is added to precipitate soluble dietary fibre and the residue is filtered, washed and dried. The residue is analysed for protein (N x 6.25) and ash, and dietary fibre is determined as the weight of the residue less protein and ash. The method has been subject to a number of modifications since its inception including most recently that by LEE *et al.* (1992) [113] and to a series of collaborative interlaboratory trials [115-120]. The gravimetric residue contains

principally undigestible polysaccharides, including some resistant starch, non-carbohydrate material including lignin, and unidentified substances [121].

Other gravimetric methods described include the urea enzymatic dialysis method of VAN SOEST [122], that of MEUSER [123] and MONGEAU and BRASSARD's technique which has been tested in two collaborative trials [116, 117].

– The enzymatic chemical methods [29, 119, 120, 124–130]

The principal method here is that of ENGLYST *et al.*, first described in 1978 [131, 132]. The method is a development of that of SOUTHGATE [99] and aims to estimate the cell wall polysaccharides as non-starch polysaccharides (NSP) in plant foods. In principle, starch is removed enzymatically after solubilisation and NSP are measured as the sum of the constituent sugars released by acid hydrolysis. A value may be obtained for total dietary fibre or, if required, soluble and insoluble fibre. Cellulose may be measured separately and the non-cellulosic polysaccharides are characterised by measurement of the individual monosaccharides by gas-liquid chromatography (GLC).

A rapid colorimetric modification of the ENGLYST method has been described [127] and an HPLC method [133–135]. The ENGLYST method has undergone a number of modifications and a series of interlaboratory collaborative trials [119, 120, 129, 130]. In practice the method measures the plant cell wall polysaccharides and chemically related (non-α-glucan) polysaccharides insoluble in 80% ethanol. Kits are commercially available for both methods.

THEANDER and co-workers [124–126] have elaborated another set of methods based on gas-liquid chromatography (the Uppsala methodology). The Uppsala and ENGLYST methods differ in two respects:

– the Uppsala method includes KLASON lignin;
– some resistant starch (retrograded amylose) is included in the Uppsala method, as in the enzymatic gravimetric methods.

Dietary fibre methods which include lignin in fact measure what is known as KLASON lignin. This is material insoluble in concentrated sulphuric acid and is not an estimate of true lignin but includes other acid-insoluble material. KLASON was a Swedish chemist who devised the method.

– Comparison of methods

Both the ENGLYST and PROSKY methods can be performed in normally equipped laboratories and give reasonably reproducible results in interlaboratory trials. Both have been applied to a wide range of foodstuffs. The major difference, as already indicated, lies in what is measured.

There have been very few formal collaborative studies in which both methods have been compared. The first study reported by the UK Ministry of Agriculture, Fisheries and Food (MAFF) in 1985 compared two early versions of the

PROSKY and ENGLYST methods [119] and amongst its conclusions was the recommendation that lignin should not be included in the estimation of dietary fibre.

A preliminary study in which three reference materials were analysed has been co-ordinated by the EC Bureau of Reference Standards. Ten laboratories used the PROSKY method [112] and satisfactory inter- and intra-laboratory variability was found enabling preliminary certified values for dietary fibre (PROSKY) to be given to these materials. However, the question as to what was being measured by the PROSKY method was not addressed. Too few laboratories used the ENGLYST method so it is impossible from this trial to get a true idea of the accuracy of the two techniques.

More recently the UK Ministry of Agriculture reported the results of an international collaborative study [120]. Thirty-seven laboratories from 12 countries took part in a comparison of the accuracy and precision of the ENGLYST GLC and colorimetric, and the PROSKY gravimetric methods for determination of dietary fibre in food. The results are summarised in **table X**.

Both methods performed reasonably well for both repeatability and reproducibility. The ENGLYST methods behaved similarly to that in earlier collaborative studies, with the GLC doing slightly better than the colorimetric method. The performance of the AOAC-PROSKY method was less good for total

Table X — Results of the MAFF trial of the ENGLYST and AOAC procedures for the measurement of dietary fibre.

Method	**Number of results** (labs)	**Fibre content** mean	**Repeatability** R-95 mean	**Reproducibility** R-95 mean
ENGLYST (GLC)				
Total (TDF)	438 (22)	8.92	1.24	2.67
Soluble	438 (22)	3.83	1.36	2.01
Insoluble	438 (22)	5.09	0.95	1.92
ENGLYST (colorimetry)				
Total (TDF)	604 (31)	9.41	1.31	3.56
Soluble	604 (31)	3.79	1.61	2.28
Insoluble	604 (31)	5.59	0.98	2.75
AOAC (gravimetric)				
Total (TDF)	282 (16)	10.82	2.11	5.34
Soluble	254 (14)	2.50	1.11	2.33
Insoluble	266 (14)	8.12	1.5	3.11

From WOOD *et al.* [120].

dietary fibre (TDF) than in earlier collaborative studies [136]. There are several possible reasons for this:

– TDF was obtained as the sum of soluble and insoluble fibre estimates, which is not the approved method;
– considerably fewer laboratories (n=14–16) performed the AOAC method than the ENGLYST methods (n=22–31);
– no enzyme kits or other support was provided for the AOAC method although kits were available commercially.

There were, however, significant differences in the results as determined by the two methods. The ratio for total dietary fibre between PROSKY and ENGLYST values was 1.19 (0.99–2.36) mean and range for the GLC method, and 1.13 (0.84–1.49) for the colorimetric ENGLYST method. Soluble fibre estimates were about 35% lower with the PROSKY than with the ENGLYST methods.

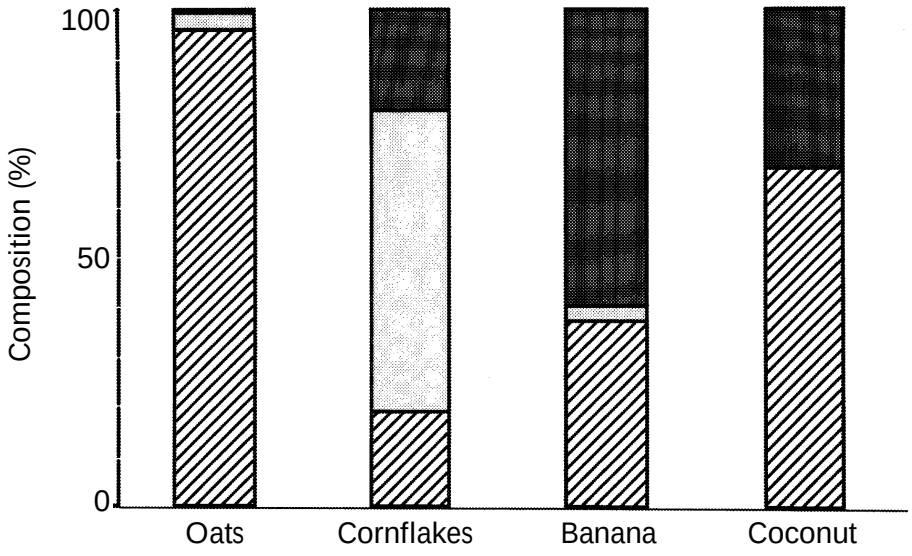


Figure 9 — Percent composition of the PROSKY residue from four foods used in the UK Ministry of Agriculture International Collaborative Trial of Dietary Fibre Methods (from ENGLYST *et al.* [121]).
■ unidentified; □ starch; ▨ NSP.

The reasons for the apparently greater values for dietary fibre as determined by the AOAC-PROSKY method are several. **Figure 9** shows that the NSP content of the residue can be very low, 19% for cornflakes and 37% for banana but as much as 96% for porridge oats. Furthermore, it is clear that the method picks up some starch which can vary from as little as 2% in oats to as much as 60% in cornflakes of the total "dietary fibre" measured. This fraction is not resistant starch as conventionally defined (see earlier). Moreover, the AOAC-PROSKY residue

comprises a variable, but sometimes large (60% in banana), fraction of material that is neither NSP nor starch. Further analysis shows that this residue is not lignin. Recent studies by ENGLYST [121] indicate that this fraction is partly MAILLARD reaction products but other unidentifiable material is picked up by the method. Food processing also affects the AOAC, tending to increase apparent fibre values.

Dietary Fibre Intakes

The amount of fibre available for fermentation can be determined from measurement of NSP in the diet. In a recent European-wide study, dietary fibre intakes were measured in 13 COST countries and the results are summarised in **table XI** [137].

Table XI — Dietary fibre intakes in Europe (g/person/day).

Country	Food balance and production statistics	Household food surveys	Studies of individuals
Austria	23.0	-	15.3–19.9
Belgium	-	-	21.0–23.3
Croatia/Yugoslavia	-	19.0–20.6	-
Denmark	21.4	19.1	22.3
Finland	23.3	-	18.4–33.1
France	25.3	15.9–17.5	13.6–22.5
Germany, GDR	22.0	17.3–20.7	-
Germany, FRG	-	-	20.3
Italy	25.0	24.7	32.0
Norway	23.0	18.0	-
Spain	-	15.1–22.4	-
Sweden	21.0	-	12.0–18.0
Switzerland	21.8	-	27.0–35.0
UK	-	11.8	11.2–12.5

From CUMMINGS and FROLICH [137].

The problem with these data is of course that a number of different methods were used for analysing dietary fibre in the various countries including the AOAC-PROSKY, ENGLYST non-starch polysaccharide and values obtained from food tables which often rely on the earlier SOUTHGATE methodology.

Data from food balance sheets and other agricultural statistics were available in nine countries. The median is 23 g/day with a range from 21 to 25.3.

The range of values is remarkably small, particularly in view of the variety of analytical methods which were used including AOAC and various gravimetric methods, ENGLYST and other enzymatic chemical methods.

Results from household food surveys were available again in nine countries. The median was 19 g/d and range 11.8 to 24.7. The result is significantly (t 2.73, $p < .05$) lower than that obtained from food balance sheets because of the nature of the method and also a greater preponderance of countries using enzymatic-chemical methods which give lower results than the gravimetric techniques. The average for the five countries using mainly enzymatic-chemical methods is 16.6 g/d and for the six using gravimetric methods 20.3 g/d. The values cannot always be divided clearly into one method or another because often food tables contain results using several different techniques, nevertheless the variance in these data is partly dependent on methodology.

In ten countries data were available on the dietary fibre intakes of individuals. These studies should approach more nearly true dietary fibre intakes but of course there are always questions about the representativeness of each group for the whole population [138]. The crude average for these studies is 20.8 g/d, with a wide range from 11.0 in the UK to 33.1 in Finland. The analytical methodologies are much more mixed here so it is not really possible to calculate averages for them with any reliance. What does emerge from the table, however, is that there is probably real variation across Europe with a range of something around 12–25 g/d using enzymatic-chemical methods and 18–30 g/d with other techniques (SOUTHGATE or gravimetric). If the data from all three survey methodologies are ranked then Italy is consistently highest for dietary fibre intake, followed closely by Finland and Croatia/Yugoslavia. At the other end of the scale are Austria, Switzerland and the UK. However, in the absence of comparable methodologies, particularly for analysis of dietary fibre, such conclusions can only be very tentative.

Physiological Properties and Health Benefits of Fibre

Much has been claimed for dietary fibre, both in terms of its physiological effects in humans and its resulting health benefits. The case for dietary fibre has probably been overstated but, now that the dust is beginning to settle on the fibre story, it is clear that at least two important properties emerge. Firstly, fibre has physical structure which it confers upon the plant foods in the diet. This results in a number of effects in man and these are summarised in **table XII**.

The other principal property of fibre relates to its non-digestibility in the upper bowel and subsequent fermentation in the large intestine by the colonic bacteria. These properties have been well described previously [139–145]. They are summarised in **table XIII**.

Table XII — Physical properties of the plant cell wall and their significance.

Property	**Physiological process affected**
Food structure	Chewing; gastric emptying; satiety; rate of nutrient release
Surface charge	Cation binding; mineral absorption; bile acid metabolism
Viscosity/solubility	Carbohydrate and sterol absorption in small intestine
Water-holding capacity	Rate of fermentation; gastrointestinal transit time; stool weight
Particle size	Rate of fermentation; gastrointestinal transit time; stool weight

Table XIII — Physiological properties and proposed health benefits of dietary fibre.

Physiological property	**Health benefit for**	**Other major contributing dietary factors**
Increased satiety	Obesity	Total energy, fat
Delayed glucose absorption and reduced insulin secretion	Diabetes Ageing	Starch
Reduced deoxycholate in bile	Gallstones	Fat, total energy, other carbohydrates
Lower blood cholesterol	Coronary heart disease	Fat, cholesterol, antioxidants
Fermentation	Large bowel cancer	Other non-absorbed carbohydrate, fat, meat
Laxation	Constipation, diverticular disease, anal conditions, irritable bowel	Other non-absorbed carbohydrates, protein degradation products

3–3 Protein and Nitrogen

The importance both quantitatively and metabolically of protein as a substrate for the flora is only just emerging. The amount of protein entering the large bowel can be partly deduced from studies of ileostomy subjects. On protein-free diets, about 1g N/day is lost in ileostomy effluent [146]. This rises to about 2 g/day when normal food is taken and is influenced by both the amount of protein in the diet and the physical form of the food [147]. A recent study showed that over a normal range of dietary protein intakes, losses into the large intestine were directly proportional to the total amount of protein in the diet and ranged from 1 g of nitrogen a day, which is largely endogenous losses, up to a maximum of around 3 g a day (about 20 g of protein) (**Figure 10**) [148].

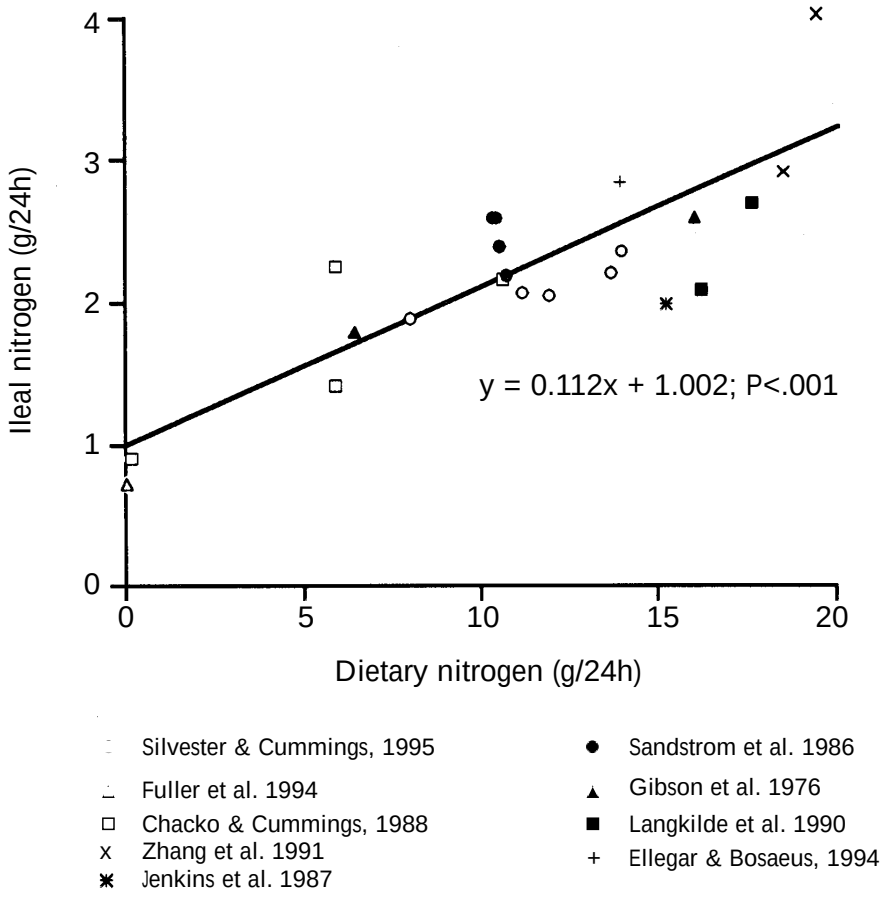


Figure 10 — Relationship between dietary nitrogen intake and ileal nitrogen excretion from nine separate studies (from SILVESTER and CUMMINGS [148]).

The majority of the N is protein (48–51%) and peptides (20–30%). Very little urea or nitrate is lost in ileostomy effluent [146, 147, 149]. On N-free diets, protein losses comprise mainly pancreatic enzymes whereas with normal food amino acid patterns in ileostomy effluent suggest that food residues are the major contributor. Surprisingly, the amount of NSP appears not to influence small bowel protein losses [147, 148, 150] except where purified polysaccharides such as pectin are given .

3-4 Mucus

Mucins are acidic glycoproteins secreted by goblet cells that line the intestinal tract where they form a protective gel covering the mucosal surface [151, 152]. Mucins have a protein core with carbohydrate side chains which contain mainly galactose and hexosamines such as N-acetylglucosamine, with some fucose [153, 154]. The carbohydrate moieties occur as linear and branched-chain oligosaccharides which constitute as much as 85% of the molecule by weight [152]. Mucins and other host-produced substances have been found to support complex communities of intestinal bacteria *in vivo* in the absence of any input of dietary substrate [155], demonstrating that they provide a source of carbohydrate for the microflora. Bacteriological studies have suggested that bifidobacteria, ruminococci, and some bacteroides play an important role in their degradation [154, 156]. Mucus is both found in ileal effluent and secreted into the large bowel. The amounts are unknown but some estimates have been made from intubation studies [80]. Mucopolysaccharides such as chondroitin sulphate also support bacterial growth [157, 158]. In total, mucus and related substances probably add an additional 3 to 5 g of carbohydrate for fermentation.

3-5 Summary

In summary, a wide variety of substrates, depending on dietary type, are available for fermentation in the colon and the range is likely to become even greater as food manufacturers use their ingenuity to bypass small bowel digestive enzymes. Overall, between 10 and 60 g carbohydrate-containing and 6 to 18 g nitrogen-containing compounds probably become available to the flora on a daily basis and constitute the major influence on intraluminal events. The nature of the diet is thus very important in determining colonic function.

4 – Short-chain fatty acids

4-1 Introduction

Both carbohydrate and protein fermentation give rise to short-chain fatty acids (SCFA) although, as we shall see later, protein is only a minor contributor to total production. SCFA are the principal products of fermentation and, through their absorption and metabolism, the host is able to salvage energy from food not digested in the upper intestine. Recent years have seen increasing interest in SCFA in human biology resulting in a number of reviews and books on the subject [8, 159–163]. SCFA affect colonic epithelial cell transport, colonocyte metabolism, growth and differentiation, hepatic control of lipid and carbohydrates, and provide energy to muscle, kidney, heart and brain. Already there are clinical uses suggested for SCFA in the management of ulcerative colitis and diversion colitis, and in enteral feeding.

4-2 Occurrence of SCFA in hindgut

Although there have been many reports of SCFA concentrations in human faeces, very few measurements have been made from material within different regions of the large bowel. **Table XIV** shows data from luminal contents obtained at autopsy from sudden death victims in the UK and also from right and left-sided colostomies of patients at Baragwanath Hospital, Soweto, who were awaiting colostomy closure some months after surgery for abdominal trauma. In all cases acetate is the dominant SCFA, and propionate is equal to or greater than butyrate. In both the autopsy and colostomy cases shown in **table XIV**, SCFA concentrations are much higher in the right side of the colon, including the caecal area. It is here that colonic bacteria first encounter carbohydrate substrates leaving the small intestine and is thus the area of highest fermentative activity. Surprisingly, however, the molar ratios of acetate:propionate:butyrate are very similar in both right and left colon. Generally, the concentrations in colostomy contents are higher but this may be because these subjects were eating a maize based diet in which starchy carbohydrates accounted for 50-60% of their total energy intake, unlike in Western countries such as the UK, where carbohydrate amounts to around 45% of energy, of which only half comes from starchy sources (mainly bread and potatoes).

Table XIV — Short-chain fatty acids in human colonic contents, in mmol/kg (molar ratios %).

SCFA	Right				Left			
	Autopsy		Colostomy		Autopsy		Colostomy	
Acetate	66.0	(52)	109.0	(62)	47.0	(52)	62.0	(56)
Propionate	26.0	(20)	36.0	(21)	17.0	(19)	30.0	(27)
Isobutyrate	1.9	(1)	0.8	(-)	2.1	(2)	1.4	(1)
Butyrate	25.0	(20)	25.0	(14)	16.0	(18)	11.0	(10)
Isovalerate	2.7	(2)	0.6	(-)	3.6	(4)	1.2	(1)
Valerate	4.0	(3)	2.1	(1)	3.5	(4)	2.2	(2)
Caproate	1.6	(1)	1.4	(1)	0.9	(1)	1.8	(2)
Lactate	3.8		-		2.3		-	
Succinate	2.0		-		2.0		-	
Number of cases	6		8		6		8	

From CUMMINGS *et al.* [164] and CUMMINGS, BEATTY and SEGAL [unpublished]

WEAVER *et al.* [165] obtained colonic contents by enema from 35 healthy subjects and found molar ratios of 60.7:15.8:17.3 (acetate:propionate:butyrate) which is broadly similar to the values in **table XIV**. Less butyrate (11.2%) was found in patients with large bowel cancer. In a study of 11 transverse colostomy and 19 sigmoid colostomy patients, MITCHELL *et al.* [166] reported higher total SCFA concentrations in the transverse region than in the sigmoid or faeces, confirming maximal fermentative activity in the right colon.

There is a remarkable sameness across the animal kingdom in the relative amounts of individual SCFA produced during fermentation. Values are very similar, both between the rumen [167] and hindgut [168] and across the different members of the animal kingdom. **Figure 11** shows molar ratios for SCFA in various species. Acetate is always the predominant anion, ranging from 55–70% of the total. In the termite it is even higher [169]. Propionate and butyrate alternate as the next most dominant fractions, with propionate in the pig and rat at 25–30% of the total, whilst butyrate is higher in the Manatee and the Dugong.

The principal substrates for fermentation in man have been detailed in Chapter 3. They include carbohydrates such as resistant starch, non-starch polysaccharides, non-digestible oligosaccharides and some non-absorbed mono- and disaccharides [27, 28, 170].

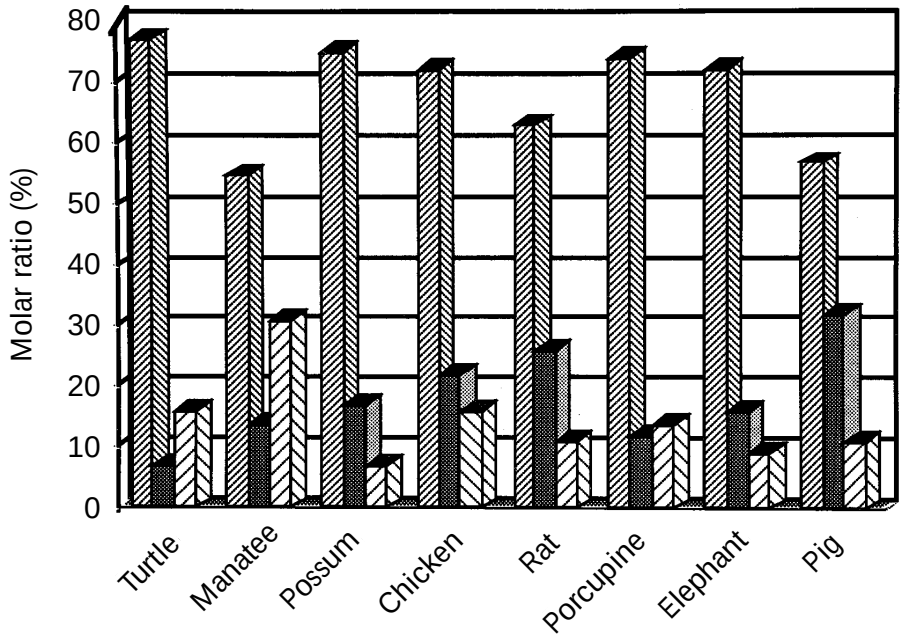


Figure 11 — Molar ratios of acetate ▨, propionate ■ and butyrate ▨ in the hindgut of a number of non-ruminant species (from BREVES and STUCK [168]).

4-3 In Vitro Studies of Fermentation

All substrates do not, however, yield equivalent amounts and types of SCFA. Although it is not easy to compare experiments done in different laboratories, **table XV** summarises eleven *in vitro* studies which have reported SCFA yields using human faecal inocula. Total yields (g SCFA/100 g substrate) vary considerably, from as low as 10 g SCFA/100 g from fibre preparations such as corn bran, pea fibre and oat fibre [171] and from cellulose, to over 60 g SCFA / 100 g for starch. Low yields may equate with incomplete fermentation, as is the case with bran NSP, or may mean other intermediates are being formed.

In all cases, acetate is the major anion comprising 67% overall of total SCFA. Pectin is a particularly good source of acetate (80% in 8 studies) whilst arabinogalactan (54%; n=3) and guar (59%; n=3) are the poorest sources. By contrast, guar and arabinogalactan are good sources of propionate (27 and 34% respectively).

Butyrate production varies over a wide range. Starch almost always gives high amounts of butyrate (62:15:23 acetate:propionate:butyrate; n=7) followed closely by oat and wheat bran (64:16:20; n=5); pectin is the poorest source

(80:12:8, n=8) along with some of the very non-digestible corn, soya, sugar beet, pea and oat sources used. With regard to overall production of butyrate, starch yields both relatively high molar proportions (23%) and has a high yield per g substrate fermented (about 50%).

Table XV — Fermentation products from carbohydrates (molar ratios %).

Substrate	**Acetate**	**Propionate**	**Butyrate**	**Yield**(1)	**n**(2)
Starch	62	15	23	49	7
Pectin	80	12	8	39	8
Wheat and oat brans	64	16	20	40	5
Other NSP	63	22	8	38	24
Mixed diets	63	22	8	38	24
Overall mean	*67*	*18*	*11*	*37*	*57*

(1) Yield= g SCFA/100 g substrate

(2) n = number of studies

From [54, 139, 171–178] and CUMMINGS, unpublished.

Other organic anions from fermentation include lactate, which is an intermediate in starch breakdown [178, 179]. Although found in the human caecum [164], lactate seldom accumulates in the colon of adults. It does, however, reach high concentrations in infants with diarrhoea. TORRES-PINEDO *et al.* [180] and WEIJERS *et al.* [181, 182] noted lactate concentrations of around 20 mmol/kg in the stools of children with carbohydrate malabsorption. However, in both studies there was also substantial acetate present, accounting for about half of the total organic anion. In children who have recovered from diarrhoea, lactate levels fall to 1–2 mmol/kg. The reason for lactate accumulation is partly its poor absorption and partly impaired further metabolism. Its major fate is oxidation by other fermenting bacteria. Lactate is produced mainly by bifidobacteria and lactobacilli. Lactate-utilising species include propionibacteria, some coliforms, and sulphate reducers. Propionibacteria and sulphate reducers grow best at near neutral pH, and then only very slowly, so they may not be able to survive in the sudden rapid transit and low pH of an infant's gut in a state of diarrhoea.

4-4 Effect of Antibiotics on Faecal SCFA

Some antibiotics impair fermentative activities in the colon and reduce faecal SCFA levels. In the studies of HOVERSTAD *et al.* [183, 184] in which groups of six healthy volunteers were given antibiotics by mouth for six days, the effects on faecal SCFA were closely related to the amounts of antibiotic which were detectable in faeces. Reductions in total SCFA (mmol/kg faeces) were seen with clindamycin (62.9 to 7.3), bacitracin (105.4 to 21.8) and vancomycin (69.3 to 19.4). Less striking changes were seen with ampicillin (62.4 to 47.8) and erythromycin (116.9 to 76.0), whilst metronidazole, co-trimoxazole, doxycycline, nalidixic acid and ofloxacin had no effect. High concentrations of clindamycin, bacitracin, erythromycin, vancomycin and nalidixic acid were found in faeces. Molar ratios of butyrate decreased with clindamycin, ampicillin, bacitracin and vancomycin.

Studies *in vitro* show that clindamycin, erythromycin and dicloxacillin reduce fermentative activity whereas penicillin and pivampicillin have no effect [185–187]. Lactate does not accumulate *in vitro* indicating a general suppression of fermentation rather than specific bacteria being affected. However, a pronounced increase in succinate concentrations in faeces (4.2 to 40.0) in healthy subjects taking a combination of neomycin, bacitracin, colistin and nystatin [188], together with reduction in SCFA (77.8 to 19.0), was seen in the studies of WILSON *et al.* [189] suggesting inhibition of succinate utilising species.

4-5 SCFA and Diarrhoea

Diarrhoea, whether due to antibiotic administration or not [187, 190–192], leads to reduced SCFA concentrations in faeces although total daily excretion may increase in proportion to rises in stool output. Studies which show increased SCFA outputs in faeces in relation to normal stool weight include the effects of bran [193] bran pentosan [194], a variety of vegetables and cereal foods [94, 95], cellulose and pectin [195] and cellulose, xylan, corn bran or pectin [196]. Similarly, in diarrhoea due to mannitol, lactulose or raffinose [190], magnesium sulphate [192] and intestinal resection [191] increased excretion with increased stool output is seen, but generally with lower concentrations.

In diarrhoea, the underlying mechanism is failure of the colon to absorb water, whether because of increased volume and solute load from the small bowel,

failure of solute absorption by a diseased mucosa, or the presence of bacterial toxins or non-absorbable ions. In any event, fermentation continues until total gut transit time falls to about 18 hours below which SCFA levels decline to very low levels [191]. Provided SCFA are being produced, and the mucosal surface is normal, the amount excreted each day will depend directly on stool weight because SCFA are the principal anion in colonic contents. Thus, measurement of their output or concentration in faeces in diarrhoea will reflect stool weight, but their molar ratios can sometimes give useful information.

4-6 Diet

At least 95% of SCFA produced in the colon are absorbed, so it is not surprising if faecal SCFA measurements prove an insensitive guide to events going on more proximally in the large bowel. This has proved to be the case particularly when studying dietary change. In FLEMING's review of 42 published studies of the effect of dietary fibre on faecal SCFA's, the majority of human studies show no effect, although in the rat, pig and monkey, some differences are seen [197]. In a recent study of starch malabsorption, induced by the α-glucosidase inhibitor acarbose, an increase in the relative and absolute amounts of butyrate in faeces was seen [198]. Similarly, faecal butyrate concentrations increased on feeding resistant starch in the form of Hylon VII to healthy volunteers [107, 108] although did not in other studies [109]. In *in vitro* studies, summarised in **table XV**, starch seems to be a good source of butyrate. Moreover starch, not NSP, is probably the principal substrate for fermentation in the hindgut of many human populations, especially those with starchy staples as the main component of their diet [27, 28] and may be important in the prevention of large bowel cancer [199] (see § 8-4-3).

4-7 Microflora

The bacteria present in the colon could theoretically make a contribution to the amount and type of SCFA present, because each genus has characteristically different fermentation end products (see **table III**). This may readily be seen when bacteria are grown in pure culture [200]. For example, *Clostridium perfringens* grown in carbon-limited cultures produces mainly acetate, butyrate, succinate and lactate, whilst *Bacteroides ovatus* produces much less acetate, a lot of propionate and some succinate. In the conditions of the colon, however, where many species

co-exist, these individual differences do not show up and a constancy of SCFA production is much more evident.

MACFARLANE and GIBSON's studies using pure cultures have, however, shown clearly that substrate availability makes a significant difference to fermentation end-product generation [200]. When *Bifidobacterium breve* was grown in carbohydrate-limited conditions, formate and acetate were the principal products, whereas with excess carbohydrate, lactate and acetate predominated. Lactate frequently appears where there is rapid fermentation of carbohydrate, and acts as an electron sink to dispose of excess reducing power, when substrate is plentiful. Similarly, with *Bacteroides ovatus,* acetate, succinate and propionate are the main products under carbohydrate-limited conditions whereas acetate and succinate predominate with excess carbohydrate. These reactions are influenced by intracellular CO_2. When sufficient CO_2 is available, as with carbohydrate-excess, there is a reduced requirement to decarboxylate succinate and therefore this acid is produced in preference to propionate [200].

4-8 Transit Time

Time is another factor which affects microbial activity in the colon. In both ruminant and human studies [201–203] bacterial growth and metabolism is much more efficient at fast turnover times, giving increased biomass yields from given amounts of substrate. This is because the maintenance energy requirements of bacteria, used for motility, active transport, and control of intracellular solute concentrations, are less at high dilution rates. *In vivo* studies in man [203] show that speeding up transit through the gut from 64 to 35 h increases biomass excretion and reduces cellulose digestibility.

Time is also reflected in patterns of SCFA production. In studies of pure cultures of *Clostridium perfringens* grown at different dilution rates, SCFA production varies. At low dilution rates butyrate was between 12 and 17% of total organic anion, depending on carbon availability, whereas at faster rates it fell to 3–8%, with lactate production increasing significantly [200]. In an *in vitro* multichamber fermentation system inoculated with mixed faecal bacteria, long retention times reduced bacterial viability, lactate and succinate production and increased acetate and propionate, especially in the first vessel of the system, which was equivalent to the right colon [204]. Long retention times also increased protein degradation when studied in a multichamber system [205], giving rise to increased concentrations of phenols and ammonia.

In the only published study in man relating transit time to faecal SCFA's [206], mean transit time (MTT) was significantly related to the proportion of

butyrate in faeces (r -0.485, p < .002). The absolute concentration of butyrate was also significantly associated with MTT (r -0.356, p < .03). No association was found between MTT and either concentration or molar ratios of acetate and propionate in this study. Thus rapid transit, in the order of 30–40 hours, is associated with a significantly higher faecal butyrate concentration and molar ratio than slow transit of 60–80 hours.

Other changes in bacterial metabolism in the colon have been associated with transit time. DAVIGNON *et al.* showed [207], in subjects on controlled diets, that there was an inverse relationship between turnover time (equivalent to MTT) and formation of the neutral-steroid conversion products coprostanol and coprostanone. An inverse relationship was also seen between turnover and degradative losses of ß-sitosterol.

In support of MACFARLANE *et al.*'s *in vitro* findings [205], longer transit times are also associated with increasing faecal ammonia concentrations and urine phenol outputs [208, 209]. Both dietary protein intake and transit time affect urinary phenol excretion, together accounting for 64% of the variation in excretion [209]. Many years ago, MACY [210] reported a similar relationship between transit and urinary sulphate excretion in children. Both the sulphate of MACY and urinary phenols are bacterial metabolites of dietary protein breakdown.

4-9 SCFA Production Rates

The importance to man of SCFA lies not only in the relative amounts of each acid produced but, more importantly, in the overall amount. Determining this is, however, much more difficult than simply measuring SCFA levels in gut contents. In ruminant animals, simultaneous catheterisation of the portal vein and an artery, together with isotope infusions, allows estimates of production to be made [211, 212]. Such studies are not really possible in man because of difficulty accessing the portal vein, and even for the ruminant physiologist they pose difficulties in interpretation because each fatty acid is metabolised by different tissues in the body, *e.g.* acetate in liver and muscle, propionate in the liver, and butyrate by the rumen epithelium (in general). The picture is further complicated by endogenous acetate production by the liver. In man, a number of approaches are possible, including direct measurement of arterio-venous (A/V) differences in SCFA concentrations across the gut at surgery or autopsy, *in vitro* fermentation models, estimation of substrate availability and breakdown, biomass production in the colon and stable isotope infusions.

4-9-1 Studies of Portal Blood

Only a very few measurements of SCFA in human portal blood have been made, and they are listed in **table XVI** [28, 164, 213–215]. These data have been acquired either at autopsy in sudden death victims (road accidents, other violent deaths, coronary heart disease) where the subject can be assumed to have been eating more or less normally until the time of death, during fasting in subjects undergoing surgery or, in surgical cases, after "feeding"—either following installation of lactulose into the colon [214] or during emergency surgery [28]. Taken together, these data give an average total SCFA concentration in portal blood of 288 mol/l, A/V differences of 192 mol/l and a daily production rate of about 277 mmol/d. If these data are separated into fasting (n = 3) and post meal (n = 2) then total concentrations are 167 mmol/l and 368 mmol/l and production rates are about 163 and 353 mmol/day respectively.

Are these SCFA arising from the gut? The presence of acetate in arterial blood allows for the possibility of production at many sites. Some endogenous

Table XVI — Short-chain fatty acid production in the human gut.

Source [ref]	**Portal blood** (µmol/l)			**Venous/Arterial** (µmol/l)			**Production**(1) (mmol/d)		
	A	**P**	**B**	**A**	**P**	**B**	**A**	**P**	**B**
UK – Autopsy [164] (non-fasting)	258	88	29	70	5	4 (V)	270	119	36
South Africa [28] – surgical (non-fasting)	271	96	56	134	16	11 (A)	197	115	65
Netherlands [213] – surgical (fasting)	114	32	9	35	2	1 (V)	114	43	11
New Zealand [214] – surgical (fasting)	128	34	18	67	4	0 (V)	88	43	26
USA(2) [215] – surgical (fasting)	133	-	-	60	-	- (A)	73	-	-

A = Acetate; **P** = Propionate; **B** = Butyrate; (A) = arterious; (V) = venous.

(1) Production: A/V difference x 1440 (min/d) ÷ 1000 (µmol to mmol)

(2) Only acetate measured

Modified with permission from Chapter 5 of GIBSON and MACFARLANE, *Human Colonic Bacteria : Role in Nutrition, Physiology and Pathology*. Boca Raton, Florida : CRC Press, 1995 [206].

(non-colonic) production does occur because subjects who have had their large intestine removed (ileostomists) have 21 µmol/l [216] acetate in peripheral blood. Moreover, during prolonged fasting (108 h) venous blood SCFA rise from 43.9 ± 4.4 (SEM) after 12 h fast to 104.6 ± 13.2 µmol/l after 60 h fast and 114 ± 15.6 after 108 h fast in healthy subjects [216]. SKUTCHES *et al.* [215] have also observed net synthesis in liver and muscle, when arterial blood levels fall below 80 µmol/l in man. Nevertheless, in the fed state the gut is likely to be the major source of SCFA.

In calculating production rates from A/V differences a number of assumptions are made. Firstly, portal blood flow over the 24 h period is estimated as 1 l/min. In practice, portal blood flow varies from less than 1 l/min during fasting to about 1.5 l/min post-prandially, but 1 l/min is a useful rule of thumb on average [217–219]. Blood SCFA levels also change between the fasting and fed state, but this variation is likely to be small since fermentation in the large intestine is a slow process and broad peak levels of SCFA are reached 8–12 hours after a meal in peripheral blood [220, 221]. Another factor to be taken into account is the partition of SCFA between red blood cells and plasma. The concentration of acetate in red cells is only 40–80% that in plasma [222, 223] and in whole blood is 80-85% of that in plasma. Of the values given in **table XVI**, all are measured in plasma except for reference [215] so a correction to production rates of 15-20% may be in order. However, portal blood SCFA measurements take no account of metabolism in the colonic epithelium. Almost certainly, 70–80% of butyrate is metabolised in the colonic epithelium, with some propionate and even a little acetate. Thus, A/V differences may underestimate total production by more than enough to compensate for partitioning between red cells and plasma.

4-9-2 Stoichiometry

Calculations from A/V differences give production rates of 300–400 mmol/day. How much substrate would be required to produce this amount of SCFA?

A number of attempts to draw up equations for SCFA production have been made [224–229] based both on rumen and human studies. Given that carbohydrate is the major substrate and SCFA, CO_2 and H_2 the principal products, the most valuable information needed to write an equation is the overall ratio in which SCFA are produced. Taking all available evidence into account, and the probable metabolism of SCFA by the epithelium, a molar ratio of around 60:20:18 acetate:propionate:butyrate can be justifiably used and gives the following stoichiometry:

$$59\ C_6H_{12}O_6 + 38\ H_20 \longrightarrow 60\ CH_3COOH + 22\ CH_3CH_2COOH + \\ + 18\ CH_3(CH_2)_2\ COOH + 96\ CO_2 + 268\ H^+$$

(Equation 1).

This avoids making assumptions about routes of H^+ disposal [230] but takes note of the fact that different substrates may produce very different relative amounts of the three SCFA when incubated alone (**Table XV**). The above stoichiometry gives a yield of SCFA from carbohydrate of 63 g SCFA/100 g. This is close to the figure of 61g SCFA/100 g carbohydrate fermented which LIVESEY and ELIA [224] suggest fits best with experimental data used to calculate the energy value of fermentable carbohydrate in mixed diets fed to humans and also with ruminant studies [201, 202, 226]. However, if the data in **table XV** are examined it will be apparent that yields of around 60 g SCFA/100 g carbohydrate fermented are seen only with starch fermentation. Cell wall polysaccharides such as arabinogalactans and pectins give yields of 35–54 g SCFA/100 g. Therefore, a theoretical yield of 63 g SCFA/100 g from the equation is either an upper limit, or starch has to be the major substrate for fermentation.

Equation 1 therefore requires 32–42 g of carbohydrate to be fermented in the human colon each day to produce 300–400 mmol SCFA. Estimates of substrate availability show that at least this amount is available for fermentation.

4-9-3 Stable Isotope Studies

Undoubtedly one of the best ways of measuring SCFA production rates in man is to use stable isotope technology. The first published attempts to do this were by WALTER *et al.* [231] investigating the sources of propionate in children with methylmalonicaciduria. Propionate production was measured by a continuous intravenous infusion of ^{13}C propionate, with breath and blood sampling for ^{13}C measurement. Total propionate production was 55–186 mmol/kg/h in the children and 17 mmol/kg/h in five healthy adults. Only 5–40% of the propionate produced in the children could be ascribed to protein catabolism, leaving a large role for colonic fermentation. Using the data of WALTER *et al.* for the children and the stoichiometry of equation 1, total SCFA production in a 70 kg man would be of the order of 380–760 mmol/d. These calculations are not possible for the healthy controls because of propionate uptake by the healthy liver.

BREVES and colleagues [232] and FREEMAN *et al.* [233] applied stable isotope technology to studies of pigs and showed that 7– 40% of maintenance energy requirements come from hindgut fermentation and that the gut itself, including the liver, may utilise a substantial portion of acetate production.

In premature infants, KIEN *et al.* [234] used ^{13}C acetate intragastric infusions, together with blood and breath sampling, to show that 24–74% of ingested lactose was fermented to acetic acid. This allows the conclusion that premature babies, who are known to digest lactose poorly, may salvage substantial energy from unabsorbed carbohydrate through fermentation.

Overall therefore, SCFA production in man has been estimated only approximately at present from static A/V difference studies across the gut or indirectly using knowledge of substrate availability, and stoichiometry derived from *in vitro*, animal and human colon fermentation studies. Net yields vary greatly depending on the type of substrate, its rate of fermentation and transit time through the colon. Stable isotope technology offers a more promising approach to this problem.

On the whole, these lines of evidence all point to a daily production in adults on Western diets of 300–400 mmol derived from fermentation of 30–40 g carbohydrate and 12 g protein. These production rates are for the Western colon and are low, contributing much less to energy needs than those observed in the pig and, particularly, in ruminant species. The amounts and types of SCFA produced in the human colon can, however, readily be increased by changing the pattern of the diet, with the introduction of more cereal based starchy foods, fruit, vegetables or other sources of resistant starch, NSP and non-digestible oligosaccharides such as inulin and other fructans. This type of diet is found in many Third World populations where SCFA production may be much greater.

4-9-4 Amino Acids as Substrates

Amino acid fermentation also gives rise to SCFA and may therefore be an additional source in man [235]. Amino acid fermentation yields not only SCFA but the branched-chain fatty acids isobutyrate, isovalerate and 2-methylbutyrate, which arise, respectively, from valine, leucine and isoleucine. **Table XIV** shows the concentration of these branched-chain fatty acids found in the human caecum. (isovalerate and 2-methylbutyrate are combined in that table because they run together in the chromatography system used). Although the amounts are much lower than the three major SCFA, the presence of branched-chain fatty acids indicates that amino acid fermentation is taking place. A fraction of total SCFA must therefore come from protein breakdown. MACFARLANE *et al.* [235] have shown, using batch culture studies of human faecal inocula, that SCFA are the principal end products formed during the degradation of protein by human colonic bacteria. Approximately 30% of protein is converted to SCFA. Branched-chain fatty acids constitute 16% of SCFA produced from bovine serum albumin and 21% of SCFA generated when casein is the substrate. Branched-chain fatty acid concentrations in gut contents taken from the human proximal and distal colons were, on average, 4.6 and 6.3 mmol/kg respectively, corresponding to 3.4% and 7.5% of total SCFA. These results suggest that protein fermentation could potentially account for about 17% of the SCFA found in the caecum, and 38% of the SCFA produced in the sigmoid/rectum. Measurements of branched-chain fatty acids in portal and arterial blood taken from individuals undergoing emergency surgery indicated that net production of branched-chain fatty acids by the gut was

in the region of 11 mmol/d, which would require the fermentation of about 12 g of protein. These data show that protein is a significant source of SCFA, and, with the known amounts of carbohydrate entering the colon, we have enough substrate available to account for 300–400 mmol SCFA production each day.

4-10 Absorption

SCFA are rapidly taken up from the human colon [236, 237] and absorption rates in the rectum, descending and transverse colon are comparable to those observed in other mammals. The first demonstration of SCFA absorption in man was from an isolated human large bowel in 1964 by DAWSON *et al.* [238]. Early mechanistic studies [236, 237, 239] showed absorption of all three SCFA which was largely unaffected by pH, with associated bicarbonate appearance in the lumen, consistent with part non-ionic diffusion and part transport of the anion.

4-10-1 General Mechanisms

SCFA absorption has been extensively studied in the rumen. They are rapidly absorbed, stimulate sodium absorption and are associated with bicarbonate exchange. pH has little effect but absorption rates are concentration dependent and show little variation due to chain length [240]. Magnesium absorption may also be stimulated [241].

In man, as in the ruminant, a large concentration gradient for short-chain fatty acids exists between the colonic lumen and blood, at around 100 mmol/l, thereby favouring their movement into blood. At the pH of caecal contents (around 5.5) [164] SCFA are largely present as anions. A transepithelial potential difference exists, with the lumen positive, also favouring absorption of the anion.

Interpretation of experimental studies is complicated by the variety of methods used to investigate absorption. Techniques include short circuit current experiments with isolated epithelia, apical and basolateral membrane vesicles and whole organ perfusion. This, coupled to the clear regional and possible interspecies differences which occur, as well as the differential metabolism of SCFA by colonic epithelial cells, makes it difficult to find a consistent pattern.

Some generalisations may, however, be made. SCFA are absorbed from the human gut and concentration dependence can be shown [237]. pH has little effect [236]. One major route for absorption is likely to be by passive diffusion of the protonated acid [242]. VON ENGELHARDT's studies [243] suggested that in the guinea pig, SCFA anions may permeate by a paracellular pathway. However, subsequent

studies in both horse and guinea pig using voltage clamping have shown that this makes no difference to unidirectional fluxes, and largely rules out paracellular transport and any associated bulk water flow. For non-ionic diffusion to occur, SCFA anions have to be protonated. H^+ is probably made available *via* a $Na^+ - H^+$ exchange in the proximal colon and $K^+ - H^+$ ATPase in the distal colon. VON ENGELHARDT, in studies on the guinea pig colon, has suggested that about 35% of SCFA are absorbed in the undissociated form in the caecum, 30–50% in the proximal colon, and 60–80% in the distal colon.

4-10-2 Bicarbonate

A relationship between SCFA and bicarbonate secretion has often been observed [236, 237, 244–249]. The consistent appearance in the lumen of bicarbonate during SCFA absorption is independent of chloride-bicarbonate exchange because it occurs in the absence of luminal chloride and is independent of chloride absorption. The amount of bicarbonate that accumulates is equivalent to about half of the acetate that is absorbed. Bicarbonate appearance in the lumen is probably the result of a SCFA-bicarbonate exchange at the cell surface.

Since SCFA transport is non-electrogenic, they must be absorbed either by anion exchange or co-transport with a cation. However, the associated changes in luminal pCO_2 and pH do not accord with a simple anion exchange. Carbon dioxide and bicarbonate in body fluids are related through the equation:

Carbonic	pKa
anhydrase	6.4

$$H_2O + CO_2 \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

(Equation 2).

Any increase in luminal bicarbonate due to secretion from the mucosa will push the reaction to the left resulting in a rise in pH and pCO_2. In experimental studies in the pig [245], rumen [250] and man [237], pH rises but pCO_2 falls during acetate absorption. The explanation that has been advanced for this is that luminal or juxtamucosal hydration of CO_2 occurs and that hydrogen ion is used to protonate SCFA anion prior to crossing the mucosa as undissociated acid. Thus, absorption of the acid leads to bicarbonate accumulation, a rise in pH and a fall in pCO_2. The colonic epithelium, in contrast to the small bowel, is rich in carbonic anhydrase [251, 252] and its inhibition reduces SCFA transport [253]. Consequently, bicarbonate secretion by the colonic mucosa, either from the cytosol or a juxtamucosal carbonic anhydrase, is important in SCFA absorption and provides the principal intraluminal buffer for fermentation.

4-10-3 Sodium

Stimulation of sodium absorption is seen in many studies of SCFA transport [237, 246, 254-257]. The stimulatory effect of fatty acids on sodium absorption is considerable. In ROEDIGER and MOORE's study [237, 246, 254-257] on the isolated perfused human colon, net sodium absorption (nmol/min/cm^2 ± SEM) increased from 320 ± 10 in the control perfusion to 1960 ± 480, with the addition of 20 mmol/l butyrate to the perfusate. In the rat colon, it is notable that neither succinate nor lactate, which are poorly absorbed anions, stimulate sodium absorption whereas acetate [247, 258] and nitrate [259] do.

VON ENGELHARDT [243] believes that interaction of SCFA with Na^+ absorption indicates that electroneutral transport of Na^+ stimulates SCFA absorption but not the reverse. Therefore, SCFA absorption itself affects Na^+ transport very little. SCFA and sodium appear to be coupled in some way and thus provide a powerful mechanism for the movement of sodium and water out of the colonic lumen. In this context, SCFA must be seen as antidiarrhoeal, and failure in their production leads to disturbances of salt and water absorption which are most notable in the germ-free animal.

Thus, SCFA absorption probably involves a number of mechanisms including both non-ionic, ionic and ion exchange processes. There are secondary effects on electrolyte transport and, perhaps most importantly for fermentation, on water movement out of the colon, and on pH through HCO_3^- and H^+ transport at the epithelial surface.

4-11 Cell Metabolism and Growth

Probably the single most important interaction between bacteria in the large intestine and their host lies in the metabolic effects of SCFA in the colonic epithelial cell. All three major SCFA are metabolised to some extent by the epithelium to provide energy, but butyrate is especially important as a fuel for these cells and may also play a critical role in moderating cell growth and differentiation.

4-11-1 Energy Metabolism

The colonic epithelium derives 60–70% of its energy from bacterial fermentation products [260, 261]. Using the isolated rat colonocyte ROEDIGER [262], subsequently confirmed by others [263–266], showed that SCFA, and

particularly butyrate, are metabolised and suppress glucose oxidation. Studies of CO_2 production using mixtures of SCFA indicate that cellular activation is in the order butyrate>propionate>acetate. SCFA are metabolised to CO_2 and ketone bodies and are precursors for mucosal lipid synthesis [267]. In the intact rat, about 12% of butyrate is transformed to ketone bodies [268] in the wall of the caecum. No ketogenesis was seen in the colon, a finding similar to that observed in the rabbit and guinea pig [264, 269, 270]. In the rabbit, studies of ^{14}C-SCFA metabolism have shown label being transferred to amino acids, carboxylic acids and sugars in the caecal wall [263, 264]. The human colonocyte also metabolises glucose and glutamine [261].

However, more than 70% of oxygen consumption in isolated colonocytes is due to butyrate oxidation, although there are regional differences. Carbon dioxide production from butyrate is similar in both proximal and distal colon in man, but ketone body appearance is less in the distal gut. This implies that more butyrate enters the TCA cycle in the distal colon and is more important in this region. Conversely, glutamine metabolism is greater proximally, as is glucose oxidation. Thus, the proximal colon more resembles the small bowel, while the distal colon relies on butyrate as a primary energy source. ROEDIGER [271] has suggested that the health of colonic epithelial cells is largely dependent on the availability and efficient metabolism of butyrate (see § 4-12-1).

Uptake and utilisation of butyrate by the colonic epithelium *in vivo* can be demonstrated from study of levels of SCFA in portal and arterial blood and in colonic contents. **Table XIV** shows the amounts and molar ratios of SCFA in material taken from sudden death victims [164] and at emergency surgery in trauma patients [28]. In the autopsy study, the molar proportion of butyrate falls from 21% within the colonic lumen to 8% in portal blood, indicating substantial butyrate clearance by the mucosa (assuming no production of acetate and propionate by the mucosa). **Table XV** shows that *in vitro* about 11% of total SCFA produced is butyrate, and *in vivo* (**Table XIV**) 11–25% of SCFA found in the colon is butyrate. The fall to 8% in portal blood indicates a clearance of 65% of the butyrate by the mucosa. If either acetate or propionate are also metabolised by the mucosa then 65% is a minimum estimate of clearance. However, the surgical data from Africa in **table XVI** show that portal blood butyrate molar ratio is 13%, whilst in colostomy contents of the patients from the same population in the same hospital, butyrate is 15% of total SCFA. This means that either the mucosa of this population is selectively metabolising acetate and propionate—an unlikely event, producing butyrate—which is equally unlikely, or that all three acids are being taken up. These data all point to substantial butyrate, and probably some propionate and acetate, utilisation by the mucosa in man.

4–11–2 Mucosal Growth

A number of studies have shown that the presence in the small bowel of dietary fibre stimulates mucosal growth and function [272–276] and likewise in the large bowel [277–281]. This trophic effect on the epithelium is almost certainly mediated by SCFA [282–286]. SAKATA has shown, by instilling a mixture of SCFA into the colon daily in rats with ileal fistulae, an increase in crypt cell production rates within two days, which was independent of luminal pH [285, 286]. Where SCFA production is reduced in either large or small bowel, epithelial cell proliferation is depressed. **Figure 12** shows results from an experiment by SAKATA [287] in which the effect of hindgut bypass on epithelial cell production, measured as crypt cell production rate (CCPR) in both large and small intestine, was measured. Bypass decreased CCPR by between 40% and 60% in all areas of the gut. This effect is also seen with low fibre diets [288, 289] and in germ-free animal studies [283].

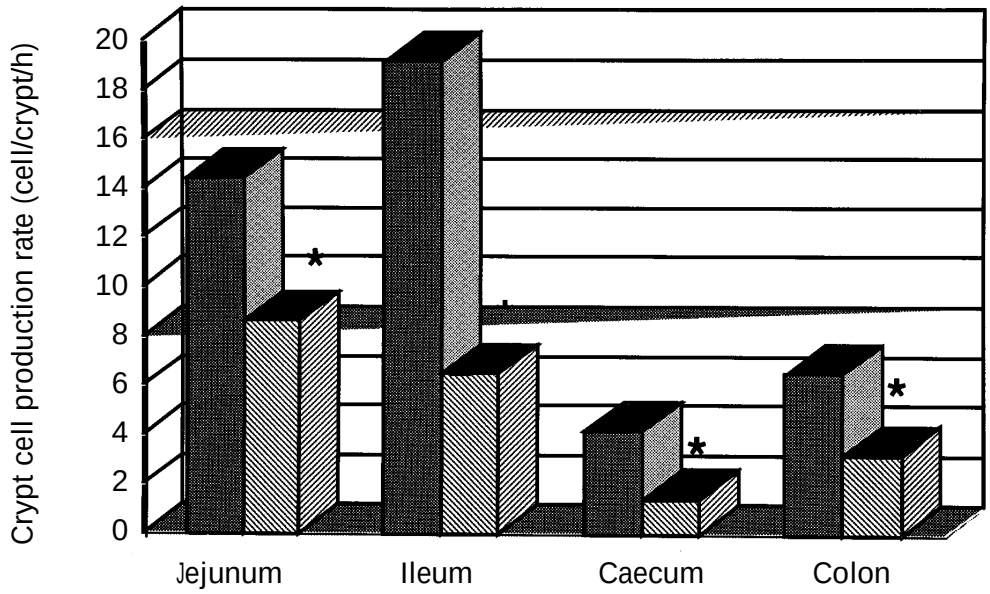


Figure 12 — Influence of hindgut bypass on the epithelial cell production rates of the small and large intestine in rats (from SAKATA [287]).
■ control; ▨ bypass.

The mechanism is intriguing, particularly the trophism which is seen in the jejunum during SCFA instillation into the colon [286]. Inert bulk is not sufficient to mediate the effect [282, 285]. ROMBEAU and colleagues have shown that the

jejunal response to SCFA occurs after either intravenous or colonic infusions [290] and that butyrate is more related to trophic effects within the colon [291]. The effect is independent of pH [290]. The effects of SCFA at a site distant from their production suggests a systemic mediator of the trophic effect. More recent studies by ROMBEAU and SAKATA [292] have shown that the mechanism may require the autonomic nervous system.

Figure 13 shows the effects both of SCFA and innervation on various parameters of intestinal mucosal growth in rats. In this model the animals underwent caecal isolation and, in addition, the caecum was denervated in one group. SCFA or saline were installed into the caecum for 10 days. In innervated rats SCFA increased jejunal DNA, villous height, surface area, crypt depth and plasma gastrin, but did not affect colonic epithelial growth. In denervated rats SCFA had no significant effect. Direct intracolonic infusions of SCFA increased mucosal DNA and crypt cell depth in the colon. Thus an intact nervous system was needed for the jejunal trophic effect. Local effects are also possible involving increases in blood flow, SCFA as an energy source or locally released growth factors.

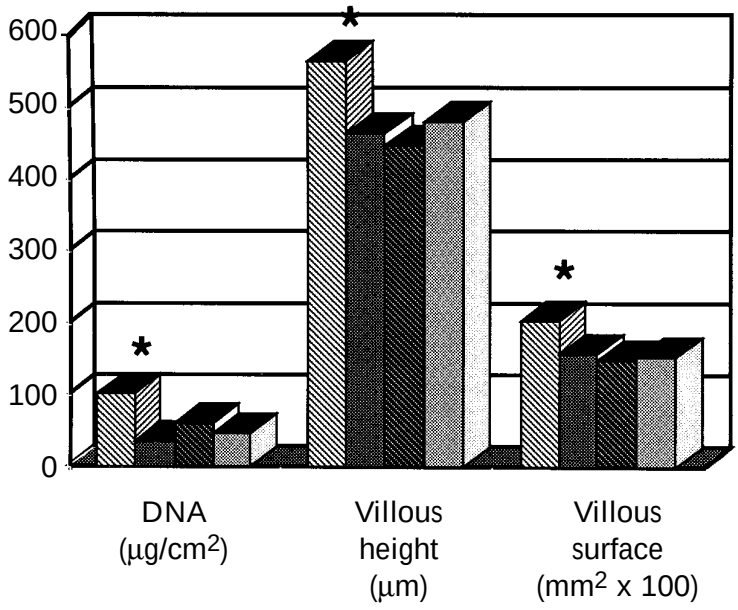


Figure 13 — Effect of the autonomic nervous system on the mediation of the trophic effects of short-chain fatty acids on the rat jejunum and colon (from FRANKEL *et al.* [292]).

innervated + SCFA; innervated + NaCl;

denervated + SCFA; denervated + NaCl.

The trophic effect of SCFA on the large bowel mucosa has led to the suggestion that it may be a factor favouring tumour development [293–295]. However, SCHEPPACH and colleagues [296, 297] have shown, with biopsy material from the human colon, that this is unlikely to be the case. Using [^{3}H] thymidine and bromodeoxyuridine to label incubated crypts, they have calculated the labelling index (a measure of crypt cell growth rate) in whole crypts and five equal compartments of the crypt. Butyrate and propionate both increased proliferation rates, whereas acetate did not. However, cell growth was stimulated only in the basal three compartments, not those near the surface as is characteristic of pre-neoplastic conditions [298]. Moreover, butyrate is well established as a growth inhibitor and inducer of differentiation in many cell lines (see § 4-12-1).

4-12 Differentiation, Gene Expression and Large Bowel Cancer

4-12-1 Properties of butyrate

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Butyrate, apart from being an important respiratory fuel for the colonocyte, has remarkably diverse properties in a wide range of cells (**Table XVII**). These are

Table XVII — Properties of butyrate in colonic cells.

- **Effects**
  - Arrest of cell growth in G1
  - Differentiation
  - Modulation of gene expression (PLAP, C fos, C-myc, CEA ...)
- **Mechanism**
  - Inhibition of histone deacetylase – increase in histone acetylation
  - Apoptosis
  - Regulation of transcriptional proteins – 5' region, SAR, HLH
- **Other**
  - Energy source
  - Membrane lipid synthesis

principally, arrest of cell growth early in G1, induction of differentiation, stimulation of cyto-skeletal organisation and alteration in gene expression. The effects of butyrate on the cell were first highlighted in 1976 in a review by PRASAD and SINHA [299, 300]. Since then a large number of papers have been written and various mechanisms of action proposed [301–307].

The slowing or arrest of cell growth is seen in many cell lines including chick fibroblasts and HeLa cells [308], ovarian cells [309], mouse fibroblasts [310, 311], hepatoma [312], bladder [313], colon [314–320], pancreas [321] cervix [322], melanoma [323, 324], neuroblastoma [325], prostate [326, 327] and breast [328]. Changes in cell growth are associated with differentiation, as indicated, for example, by the expression of alkaline phosphatase activity. KIM and co-workers [329–331] have shown that in normal colonic mucosa, intestinal alkaline phosphatase activity is increased by butyrate, whilst treatment of tumour tissue produces placental-like alkaline phosphatase (PLAP). They have also shown that butyrate induction of PLAP in colon cancer cells is regulated by increased message levels which occur with differentiation. In LS174T cells, the 5' flanking regions of the PLAP gene were found to contain cis-acting elements which regulate PLAP expression. The suppressor effect for gene expression of the PLAP promoter was removed by butyrate, suggesting that butyrate-induced factors lead to the release of a repressor band in this region before induction [331]. The importance of specific DNA sequences in butyrate sensitivity of particular genes has also been shown for two cytotoxic cell protease genes from T-lymphocytes [303] and the embryonic globulin gene [305].

The effect of butyrate on differentiation is therefore related to the control of gene expression. TOSCANI *et al.* [311, 332] have shown with Swiss 3T3 cells that the arrest of cell growth and differentiation is not a generalised shutting-off of the expression of growth-associated genes, but rather a specific reduction of c-myc, p53, thymidine kinase and induction of c-fos and aP2. These events ultimately lead to adipocyte differentiation in Swiss 3T3 cells, when combined with either insulin or dexamethasone.

Butyrate alters the expression of many genes, including the induction of hemoglobin synthesis in murine erythroleukemia cells [333], EGF receptors [334–336] in hepatocytes, plasminogen activator synthesis in endothelial cells [337], thyroid hormone receptors in the pituitary [338], metallothionein in hepatoma cells [339], estrogen, prolactin and EGF receptors in breast tissue cells [340–341] and many others. In colo-rectal cancer cells a number of changes in gene expression have been observed including induction of c-fos [342], PLAP [343] and carcinoembryonic antigen [344], inhibition of urokinase and release of plasminogen activator inhibitor [345, 346], expression of brush border glycoprotein [320] and P-glycoprotein phosphorylation [347]. The induction of differentiation in tumor cell lines is associated with changes in cytoskeletal architecture and adhesion properties of cells [348–350].

4-12-2 Cellular Mechanisms

KRUH suggested a number of cellular mechanisms for the action of butyrate [302, 351]. The best known is the effect on histone acetylation which has been shown in many cell types. SMITH [304] has demonstrated that by inhibiting histone deacetylase, butyrate allows hyperacetylation of histones to occur. In turn, this "opens up" DNA structure, facilitating access of DNA repair enzymes. He has grown human adenocarcinoma cells (HT29) at a range of butyrate concentrations up to 10 mmol/l and shown inhibition of cellular proliferation, which was approximately concentration-dependent. Cells were blocked in G1 phase. Cells grown in 5 mmol/l butyrate and exposed to various DNA damaging agents showed increased resistance to ultraviolet radiation damage and to adriamycin but, surprisingly, increased sensitivity to x-irradiation. Accessibility of DNA to endonucleases was increased and a compensatory increase in ultraviolet repair incision rates seen. In an animal study in which wheat bran was fed, BOFFA *et al.* [352] demonstrated that butyrate levels in the colonic lumen are positively related to colonic epithelial cell histone acetylation and inversely related to cell proliferation. Butyrate thus appears to be able to modulate DNA synthesis *in vivo*.

However, increase in histone acetylation may not be the entire explanation for the specificity of butyrate in modulating gene expression. Evidence is now accumulating that butyrate acts by a mechanism that involves specific regulatory DNA sequences. GLAUBER and colleagues [305] have shown, with adult erythroid cells, that 5' flanking sequences upstream of the embryonic p-gene are a major determinant of p-gene expression and mediate the stimulatory action of butyrate on p-gene transcription. Similarly, in T-lymphocytes, FREGEAU *et al.* [303] have shown a butyrate sensitive region in the 5' flanking sequence of a cytotoxic cell protease gene. A specific effect of butyrate on transcriptional regulatory proteins and promoter regions seems likely [331, 342, 353]. Furthermore, KLEHR *et al.* [354] have shown that the stimulatory effect of butyrate on gene expression is greatest if one, or especially two, scaffold/matrix-attached regions are present adjacent to the gene. They suggest that butyrate induced inhibition of histone deacetylase may have consequences for chromatin structure which would, in turn, promote transcription.

Other possible mechanisms of action suggested by KRUH include inhibition of chromatin protein phosphorylation, hypermethylation of DNA and chromatin structure. Recently BUSTIN has shown that butyrate moderates the expression of two related genes BRF1 and BRF2 (butyrate response factor genes) which are members of the TIS 11 family of primary response genes, and are associated with the control of neoplastic growth [355, 356]. PARASKEVA and colleagues [306, 357] have suggested that butyrate causes apoptosis. In cell lines from colorectal cancers and polyps, sodium butyrate in 1–4 mmol/l concentrations induced apoptosis,

while TGF_{α} did not. Apoptosis in the colon may therefore be triggered as cells migrate up the crypt and are exposed to luminal growth factors.

Does all this convert into a mechanism whereby anaerobic bacteria in the gut produce, from dietary carbohydrate, factors which lead to protection from large bowel cancer? Butyrate can induce transformed cells to acquire the phenotype of more differentiated cells but, as YOUNG and GIBSON point out [320, 358], butyrate has paradoxical effects on normal and transformed colon epithelial cells. Some of the conflicting results in the literature may be related to the concentration of butyrate used in the *in vitro* studies. The most effective concentrations for inducing differentiation are usually no more than 5 mmol/l whilst high concentrations lead to cell death. However, luminal concentrations of butyrate in the human colon can exceed 20 mmol/l (**Table XIV**) and are usually in the range 10–30 mmol/l. It is difficult to believe that a naturally occurring fatty acid, ubiquitous in the mammalian hindgut, could promote or select for tumour growth. Its origin from fermentation of dietary NSP and RS does however provide a link between epidemiological studies which show these carbohydrates to be protective against large bowel cancer and the cellular mechanisms that occur.

In the 14 years that have elapsed since it was first proposed that butyrate was the link between fibre and protection against large bowel cancer [359], it has remained the most likely candidate to fulfil this role. In addition, it is an important energy source for the colonic epithelium, substrate for lipid synthesis and its production by bacteria is therefore of major importance to health.

4-13 Metabolism of SCFA

4-13-1 Acetate

Acetate is an essential metabolic fuel in ruminant animals because all glucose reaching the rumen is fermented by the resident bacteria. In man, its importance is less certain because it derives from the hindgut and as such must represent a secondary fuel for the tissues, after carbohydrate absorbed from the small bowel. Acetate is always found in human venous blood [215, 216, 220] with fasting levels around 50 μmol/l rising to 100–300 μmol/l after meals containing fermentable carbohydrate. Oral metronidazole has little effect, despite suppressing hydrogen and methane production [216]. Acetate is rapidly cleared from the blood with a half life of only a few minutes [215, 360] and is metabolised by skeletal [216, 361] and cardiac muscle [362, 363] and brain [364]. Blood acetate is derived primarily from the gut [216, 365]. Patients who have no large intestine have very low blood acetate levels. However, liver synthesis probably occurs

when blood levels fall below a certain critical level [215, 366, 367], as seen in starvation for example [216]. Acetate spares fatty acid oxidation in man. Free fatty acid levels fall when oral acetate or alcohol, which is an immediate precursor of acetate, is given [361, 367, 368]. Acetate either orally or intravenously has little effect on glucose metabolism and does not stimulate insulin release in man [369, 370].

4–13–2 Propionate

Propionate metabolism has been extensively studied in ruminants where it is a major glucose precursor [371, 372]. Much less is known about its role in man. Propionate can be found in portal blood, although some may be metabolised in the colonic epithelium and may be a differentiating factor, but with less power than butyrate [314]. Propionate supplemented diets have been shown to lower blood cholesterol in rats [373, 374] and pigs [375–378] but in man the effects are less clear. In a double-blind placebo controlled study of ten female volunteers fed 7.5 g sodium propionate daily for seven weeks [379], there was no change in serum cholesterol but HDL cholesterol increased as did triglycerides. There were falls in fasting blood glucose levels and maximum insulin responses to standard glucose loads. In a similar study, lasting one week, again no effect on cholesterol was seen but glucose levels were lowered [380]. In another study nine healthy males, whose initial cholesterols were all > 5.5 mmol/l, were given 5.4 g propionate daily for 15 days and showed a fall in total cholesterol (to 5.28 ± 0.23 mmol/l) and in LDL cholesterol, whilst acetate was without effect [381].

To overcome the objections that oral propionate does not reflect the true physiology of propionate produced by fermentation in the hindgut, two studies using rectal or colonic infusions of propionate have been undertaken [382, 383]. Propionate had no effect or slightly raised serum cholesterol in short term studies. Other experiments have shown inhibition of hepatic cholesterol synthesis by propionate and redistribution of cholesterol from plasma to liver [373, 374, 384]. There is also extensive hepatic metabolism of SCFA [385].

Thus SCFA have many roles in the human large intestine and in metabolism. Following on from this, a number of clinical uses are developing including incorporation of SCFA into parenteral infusions during bowel rest and post-surgical procedures involving gut anastomoses. They also have a role in ulcerative colitis and diversion colitis (see Chapter 6).

5 – Hydrogen metabolism

5–1 Introduction

Gas production is an integral part of fermentation, and the presence of CO_2, H_2 and CH_4 has been well documented in the human large intestine.

Figure 14 shows the average composition of intestinal gas from the studies of LEVITT [386]. Traces of oxygen, up to 1%, may also be found. Considerable variability has been observed in the composition of flatus gas; for example, CH_4 has been reported to reach 29% on occasions [38, 386–390].

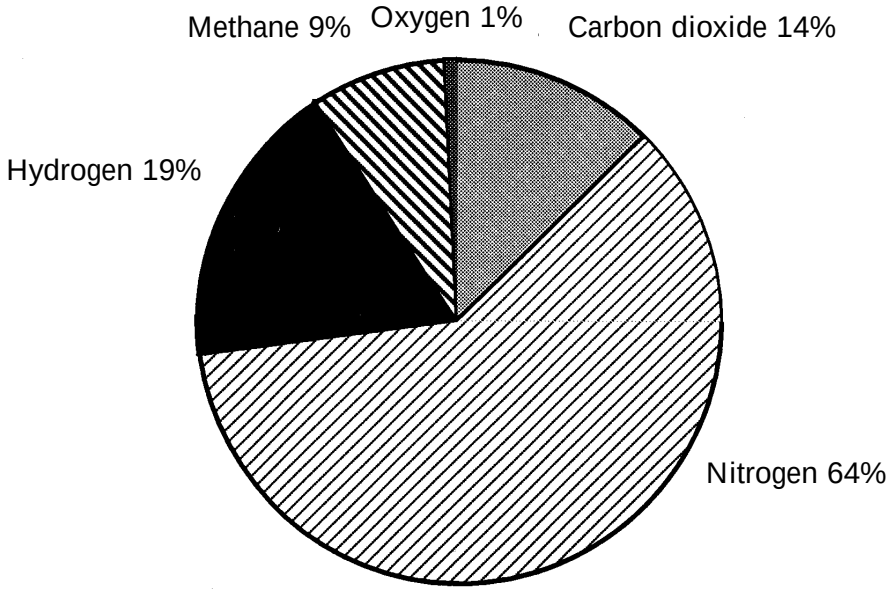

Figure 14 — Composition of intestinal gas. The total percent of gases given in the figure to 107%. This is because the value of 9% for methane relates to only four the eleven subjects which LEVITT *et al.* studied. Overall the methane value for the whole group was 3% (from LEVITT [386]).

The total amount of gas produced each day from fermentation varies, mainly in relation to diet, with values of 0.5–4 l/d being reported [38, 386–392]. A number of studies show that dietary intake of foods such as beans [388, 389,

393], some fruit juices [394], cereal fractions [395–397], Brussels sprouts [389], some starches [392, 398] and dietary fibre [399, 400] substantially increases gas production, as does consumption of non-absorbable sugars such as lactitol and lactulose and other low molecular weight carbohydrates [39, 389, 401, 402].

The precise stoichiometry of intestinal gas production in man is unknown at present, but for rapidly fermented sugars, values as high as 45–50 ml H_2 per g carbohydrate have been reported [402, 403]. Hourly production of flatus gas is usually less than 100 ml but has been reported to rise to 168 ml in subjects ingesting 51% of their calories as beans [393] whilst a rate of 346 ml/h was recorded by LEVITT *et al.* [403] in a patient with lactose intolerance. In this subject, CO_2 and H_2 comprised 72% of total flatus gas at peak production times. Flatus gas is passed about 14 times a day in healthy subjects, with a volume of between 25 and 100 ml on each occasion [387, 403]. A significant and variable proportion of all gas produced in the large gut is also absorbed and excreted in breath. The major gases present in breath samples are H_2 and CO_2, as well as CH_4 in some individuals [404–407]. Low amounts of mercaptans (methanethiol, ethanethiol) [408], ammonia [409] and H_2S [387] can also be detected.

Carbon dioxide from fermentation equilibrates with the body bicarbonate pool and is only a very small contributor to breath in CO_2. Hydrogen, however, is unique to fermentation and may be excreted via a number of routes (**Figure 15**).

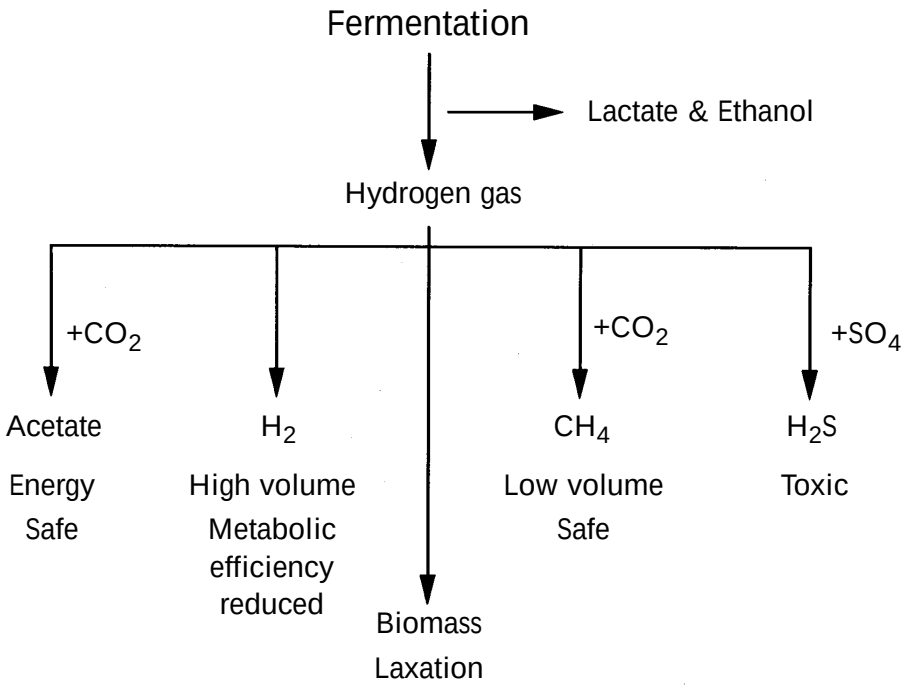

Figure 15 — Principal pathways of reducing equivalents generated from fermentation in

5-2 Hydrogen H_2

In the colon, H_2 is formed by bacteria to dispose of reducing equivalents generated during fermentation. Its formation is largely a result of the oxidation of pyruvate, formate, reduced pyridine nucleotides (NADH, NADPH) and reduced ferredoxins. However, high partial pressure of H_2 in some anaerobic ecosytems may have the effect of lowering the efficiency of fermentation by inhibiting re-oxidation of the reduced co-enzymes [227, 410].

The excretion of H_2 in breath has been used as a quantitative index of carbohydrate fermentation in man [411–414]. These studies assume that the proportion of H_2 produced is constant, irrespective of the chemical composition of the fermentable substrate, and that breath and flatus excretion are linearly related. However, the data of CHRISTL *et al.* [392] show that this is not necessarily the case. In this study a whole body calorimeter was used to measure H_2 and CH_4 excretion in ten volunteers, during a 36 h period, in response to varying amounts of lactulose, pectin and starch.

Figure 16 gives the results for hydrogen generation from lactulose, pectin (a non-starch polysaccharide) and starch from banana (RS2). Nearly four times as much hydrogen was excreted per gram of lactulose fermented compared to starch or pectin.

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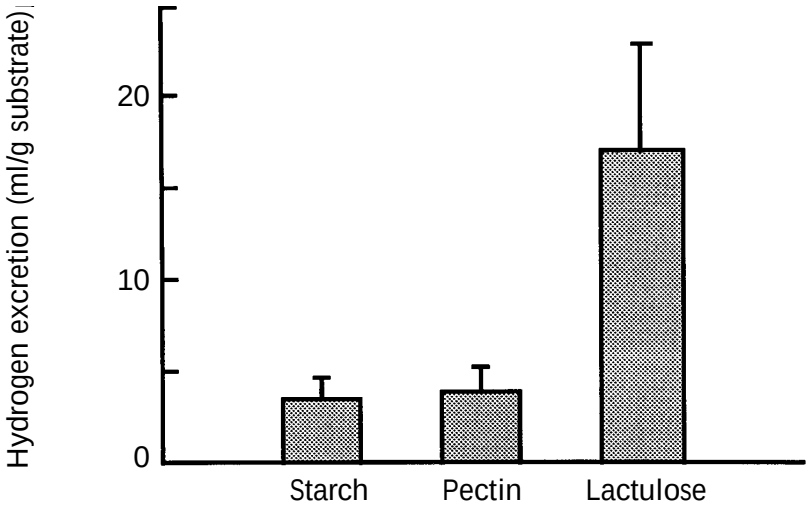

Figure 16 — Hydrogen excretion from ten healthy subjects given either 15 g of lactulose or 20 g of pectin or 250–350 g of banana containing 23 g of resistant starch (modified from CHRISTL *et al.* [392]).

There are a number of possible reasons for this. As each carbohydrate requires a variety of bacterial enzymes for its breakdown [228, 415–417], a distinct consortium of bacteria will be best capable of fermenting it. The metabolic pathways preferred by these bacteria lead to different patterns of products. In vitro fermentation studies have shown that various polysaccharides yield different proportions of the main end products, short-chain fatty acids (**Table XV**). During fermentation to more oxidised products, such as acetate, H_2 is generated as an electron acceptor while in the formation of more reduced products such as propionate net hydrogen consumption occurs.

In the same study of CHRISTL *et al.* [392], two other important facts emerged. Firstly that, on average, 58% of H_2 gas produced was excreted in breath, but this value was variable (range 25–65%), depending on whether low (< 200 ml/d) or high (> 500 ml/d) production rates occurred. These data are summarised in **figure 17**. At low rates of hydrogen or methane production (up to 200 ml/d) 65% of these gases were excreted in breath. Over this the proportion decreases to 25% in breath. This is explicable on the basis that at higher production rates the gas is

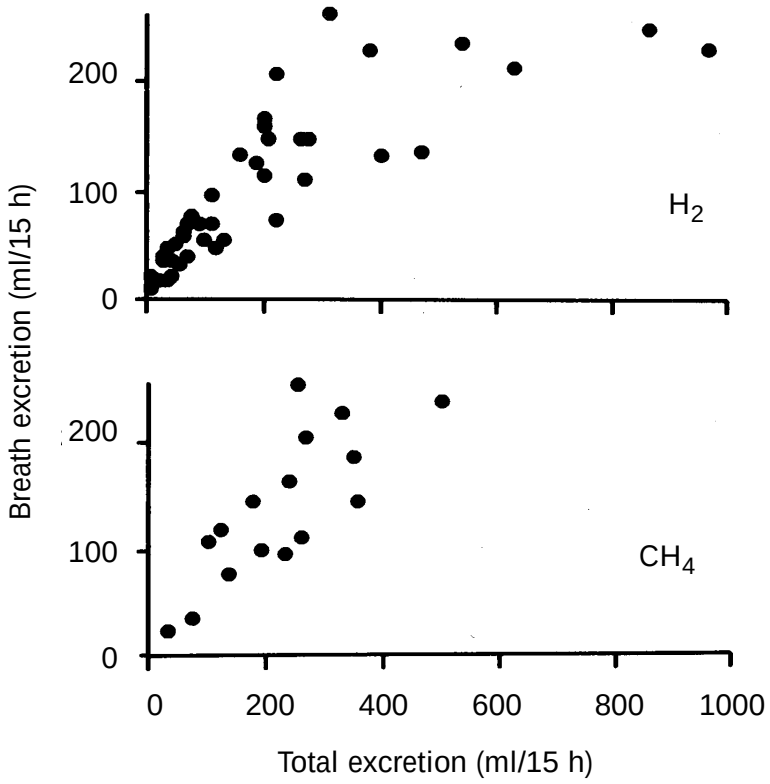

Figure 17 — Hydrogen and methane excretion in breath *versus* total excretion in ten healthy subjects taking varying doses of lactulose (from CHRISTL *et al.* [392]).

more likely to accumulate in significant amounts in the colon and be expelled as flatus as a result of stimulating motor activity. At low rates it can diffuse into the blood fast enough not to accumulate to any extent.

Perhaps the most important finding from these studies was the very small amount of hydrogen collected compared to the theoretical amounts expected from the stoichiometry (see equation 1 in Chapter 4). **Figure 18** shows that for the three doses of lactulose only 5% of the expected hydrogen was collected over 24 h. When account was taken of methane excretion as well, still only about 10% of total possible hydrogen was accounted for. This is because there are a number of alternative pathways for hydrogen disposal or utilisation other than excretion as

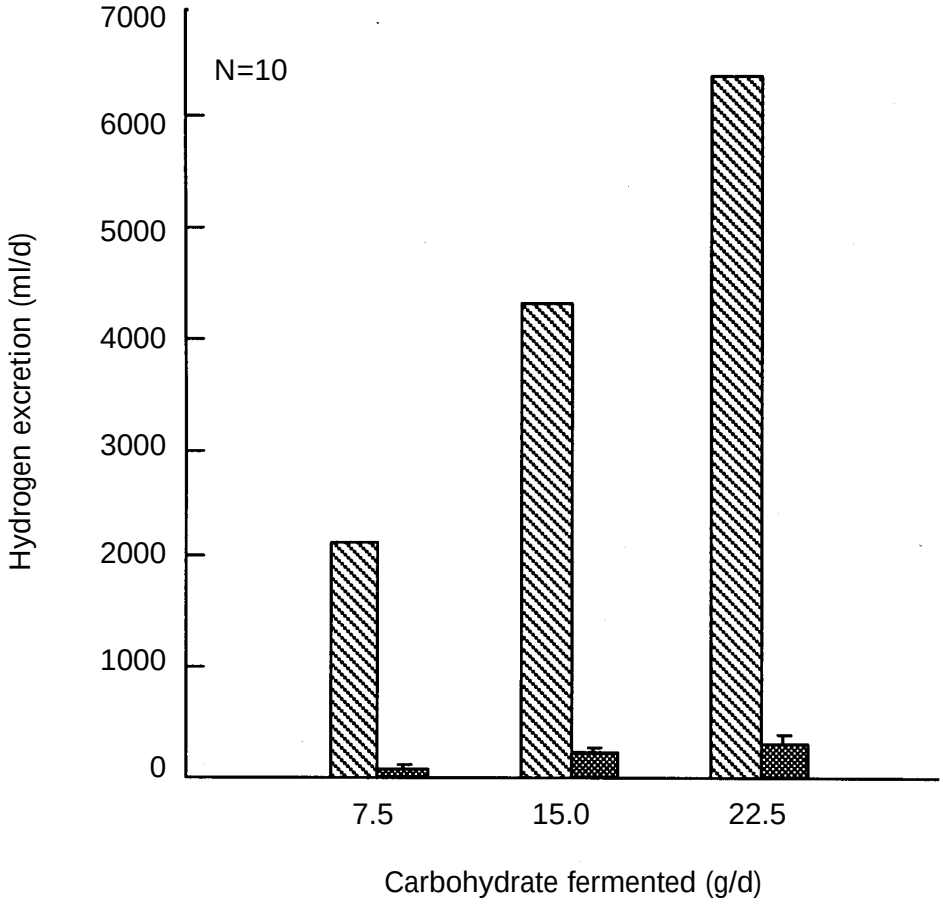

Figure 18 — Total hydrogen excretion in ten healthy subjects after three different doses of lactulose compared with theoretical hydrogen production from that amount of fermented carbohydrate (from CHRISTL *et al.* [392]).
▨ theoretical excretion; ▩ actual excretion

hydrogen gas. As **figure 15** shows, these include the formation of methane, reduction of sulphate to sulphide, acetogenesis, lactate and ethanol formation and, perhaps most importantly, the incorporation of reducing equivalent into biomass.

All of these observations make the interpretation of breath hydrogen, a widely used test in gastroenterological clinical practice, difficult to interpret. The amount of hydrogen that appears in breath in relation to the grams of substrate fermented will vary depending on the type of substrate, on the disposition of hydrogen excretion between breath and flatus and on the activity of the various alternative pathways of hydrogen disposal.

Breath hydrogen is also used as a transit marker in gastrointestinal studies, using lactulose as the fermentable carbohydrate source [418]. The interval between ingestion of lactulose and first appearance of H_2 varies between 30 and 100 min and is related to the dose used; larger doses giving faster mean transit times (MTT) [418, 419]. Similarly, higher molecular weight substances also give slower apparent MTT [399]. It is not therefore a true measure of transit time, and other methods are required to determine the average rate of passage for complete meals to the caecum [420, 421]. A small proportion (about 5%) of subjects do not produce any detectable H_2 in breath following ingestion of fermentable carbohydrate [422, 423] and this may be related to changes in colonic luminal pH during fermentation [424].

5-3 Methane

CH_4 formation in man is altogether more enigmatic than H_2 production. In studies of Western populations, only 30-50% of healthy subjects produce detectable CH_4 in their breath [401, 404, 425–429]. Children under the age of two do not excrete CH_4, although the proportion of producers gradually rises until adult levels are reached at about the age of ten, and there is a strong association in CH_4 excretor status amongst family members [407, 430]. An unexpectedly high breath CH_4 level occurs in patients with large bowel cancer. Between 80% and 90% of these patients have detectable CH_4 in breath compared with only about 40% of controls. Resection of the tumour causes the frequency of excretion to return to normal healthy population levels. Moreover, the proportion of CH_4 producers is also increased in patients with pre-malignant bowel conditions such as polyps [425–427, 431]. Certain pathological conditions of the large intestine would therefore appear to promote CH_4 production. The presence of haem has been suggested as an important factor, but luminal haemoglobin in the presence of tumour does not appear to influence CH_4 excretor status [432].

Methanogenic bacteria (MB) in the large bowel have an obligate requirement for H_2. The principal CH4-producing species is *Methanobrevibacter smithii* which reduces CO_2 with H_2, according to the following equation [155, 433–435]:

$$4H_2 + CO_2 \longrightarrow CH_4 + 2\ H_2O.$$

Methanosphaera stadtmaniae can also be isolated from some faecal samples and this species combines methanol with H_2 as shown in the equation [434, 436]:

$$H_2 + CH_3\ OH \longrightarrow CH_4 + H_2O.$$

In the rumen, methanogenesis has an important effect on the end products of fermentation, by removing H_2 produced from reduced pyridine nucleotides. This removal of H_2 results in an increase in the formation of more oxidised metabolities such as acetate and a decrease in propionate [437]. In contrast to the rumen, methanogenesis does not appear significantly to affect SCFA production in man. Although high concentrations of ethanol may be detected in the proximal colon, lactate and succinate do not accumulate in the large gut, and the molar ratios of acetate, propionate and butyrate are similar in methanogenic and non-methanogenic individuals [225, 438]. In this case, the operation of alternative methods of hydrogen disposal may be of some significance (see below).

Unlike H_2, CH_4 production does not seem to change perceptibly with variations in diet [389, 393, 400, 404, 422]. Intestinal intubation and sudden death victim studies indicate that CH_4 production predominantly occurs in the distal colon [26, 439].

From the host viewpoint, the reduction of 4 mol of H_2 to produce 1 mol of CH_4, which is relatively harmless and easily expelled, may be of some clinical importance from the viewpoint of gas accumulation in the colon.

5-4 Sulphate and Sulphide

An alternative pathway for disposal of the H_2 generated from colonic fermentation is through the activities of dissimilatory sulphate-reducing bacteria (SRB). They utilise H_2 according to the equation :

$$4\ H_2 + SO_4^{2-} + H^+ \longrightarrow SH^- + 4\ H_2O.$$

The growth of SRB in the presence of sulphate, reducing it to sulphide, is the principal taxonomic characteristic of SRB, although the genera themselves are biologically diverse and quite unrelated to each other. SRB are able to multiply in anaerobic regions of marine and estuarine sediments and muds derived from brackish water. SRB can also be found in the intestinal contents of humans and

experimental animals, and were first isolated from human faeces in 1977 [440], the principal genus being *Desulfovibrio*, accounting for 64–81% of all SRB isolated in more recent studies [230, 441, 442]. These strains are able to use gaseous hydrogen as an electron-donor for the reduction of sulphate to sulphide, and account for substantial consumption of hydrogen within the colon. SRB have a preference for growth in the distal colon, shown initially in an *in vitro* model of the colon, using a three-stage continuous-culture system [443] and confirmed *in vivo* at autopsy [26]. Levels of luminal sulphide are maximal in the rectosigmoid region [26, 441], and may help explain the predilection of ulcerative colitis for the distal colon.

GIBSON and colleagues [158, 444, 445] have shown that sulphate-reducing bacteria occur in the human gut and are carried principally by people who do not excrete methane in breath [441, 446]. In *in vitro* studies, SRB outcompete methanogens for H_2 [158]. SRB require sulphate as an electron acceptor and produce SH^-. Feeding sulphate to healthy methanogenic individuals causes inhibition of methane excretion and the growth of SRB in those subjects carrying low numbers of SRB initially [447]. *In vitro,* SRB growth is modulated by sulphate availability even in the form of mucus [441–443].

Faecal samples donated by healthy volunteers living in the United Kingdom and South Africa have been used to determine interactions between colonic SRB and MB [442, 448]. Although the Africans were predominantly CH_4 producers, a strong inverse relationship between methanogenesis and SO_4^{2-} reduction was found to occur in samples from both populations. Subsequent studies with faecal slurries confirmed that the bacteria competed for the mutual growth substrate H_2 [158]. In methanogenic individuals, H_2 did not accumulate during 48h *in vitro* incubation of faecal slurries—however, significant CH_4 production occurred. In their non-methanogenic counterparts, H_2 levels again remained low, but H_2S was a major product. Mixing of the two types of slurry demonstrated that colonic SRB were able to directly outcompete MB for the available H_2. Although many different electron donors are potentially available in the large gut to serve sulphate reduction, species belonging to the genera *Desulfovibrio* and *Desulfobulbus* are particularly efficient H_2 utilising bacteria [444]. In a competitive environment such as the colon where H_2 is a limiting substrate, SRB are able to lower the partial pressure of H_2 to levels below those at which methanogens are able to effectively compete. For this process to occur, a sufficient supply of electron acceptor (SO_4^{2-}) must be available. Pure culture studies have shown that SRB have a much greater substrate affinity for H_2 than methanogens (for *Desulfovibrio vulgaris* Ks = 1 mmol/l; for *Methanobrevibacter smithii* Ks = 6 mmol/l) [449].

The critical role of SO_4^{2-} availability in this relationship was demonstrated

by CHRISTL *et al.* [447]. In this study, six methanogenic volunteers were fed a SO_4^{2-} enhanced diet for 10 days (15 mmol/d). During this time, SO_4^{2-} reduction and methanogenesis were continuously monitored and compared with control periods (10 days) either side of the test. In half of the volunteers, the addition of SO_4^{2-} resulted in a rapid decrease in methanogenesis and concomitant stimulation of dissimilatory SO_4^{2-} reducing activities. In the other persons, however, the increased availability of SO_4^{2-} had no effect on CH_4 production. These studies suggest that at least two types of methanogenic person exist: one group harbours populations of SRB that are apparently inactive and probably limited by a reduced supply of electron acceptor, although these bacteria are able to grow and outcompete MB for H_2 when SO_4^{2-} becomes available. In the other group, SRB are absent. Concentrations of SO_4^{2-} vary markedly in diet (range 2–9 mmol/d) [450]. Foods with a high sulphate content include fermented beverages and dried fruits [451]. Endogenous sources of SO_4^{2-} may also make some contribution to the available pool. For example, some host secretions contain highly sulphated glycoproteins, particularly colonic mucins, which are extensively degraded by the gut flora to release this anion in a free form [156, 443, 452–455].

Dissimilatory sulphate reduction is a clinically undesirable method of hydrogen disposal. The common end product of SRB metabolism, H_2S, is highly toxic to the colonic mucosal barrier, and impairs cellular metabolism [456, 457]. Coincidentally, the majority of gut disorders arise in the distal colon, where highest numbers and activities of SRB occur .

From the foregoing, it is clear that an inverse relationship between SO_4^{2-} reduction and methanogenesis exists in the large bowel. SO_4^{2-} availability undoubtedly plays a key role in hydrogen utilisation. However, other factors such as pH, bacterial distribution in the colon and nutritional status of the individual are also likely to be significant.

5-5 Acetogenesis

The situation is further complicated by the existence of other potential pathways of H_2 disposal by colonic bacteria [448]. For example, homoacetogenic bacteria also grow in the large gut [458, 459]. These organisms are able to combine 4 mol of H_2 with 1 mol of CO_2 to produce acetate. Energetically, however, acetogenesis is a less favourable route of hydrogen disposal than either sulphate reduction or methanogenesis and acetogenic bacteria, under normal circumstances, are outcompeted by SRB or MB for H_2. Thus, significant levels of

acetogenic activity would only be expected when conditions unfavourable for methanogenesis or sulphate reduction occur.

Gas production in the large bowel is frequently of some concern to patients with digestive disorders, or diseases such as pneumatosis cystoides intestinalis [460]. The underlying mechanisms that control both gas formation and utilisation in the gut remain unsolved. However, it is clear that the colonic microflora plays a significant role and that gross physiological differences can occur both between and within distinct populations.

5-6 Pneumatosis Cystoides Intestinalis

Pneumatosis cystoides intestinalis (PCI) is an uncommon condition characterised by the presence of multiple gas-filled cysts in the mucosal lining of the large intestine that contain hydrogen, nitrogen and carbon dioxide [461, 462]. Patients complain of diarrhoea with mucus and of passing excessive amounts of gas. Breath hydrogen concentrations are usually elevated in these patients compared with normal subjects [463, 464].

Acumulation of hydrogen in the colon may be caused by either an increased fermentative H_2 production or by a reduced removal of this gas. In order to determine which was the mechanism in PCI, CHRISTL *et al.* [460], measured total H_2 excretion in the calorimeter in two patients and breath H_2 concentrations in three patients with PCI while they were taking a diet free of fermentable carbohydrates (**Figure 19a**) and then in response to a dose of lactulose (**figure 19b**). The presence and metabolic activity of the major H_2-consuming bacteria in faeces were also determined and the data were compared with results from ten healthy volunteers.

H_2 excretion rates in ml/min were grossly elevated both in the fasting state and in response to lactulose. Total H_2 excretion in patients was 383–420 ml/d on the basal diet and 1430–1730 ml/d after lactulose administration compared with 35 ± 6 ml/d and 262 ± 65 ml/d, respectively, in controls. Basal breath H_2 levels in controls were 27 ± 6 *vs.* 214 ± 27 ml/d in patients and, after lactulose ingestion, 115 ± 18 *vs.* 370 ± 72 ml/d. Four controls were methanogenic and had high faecal MB counts. The other controls had high SRB counts and sulphate reduction rates. All patients were non-methanogenic and had low sulphate reduction rates.

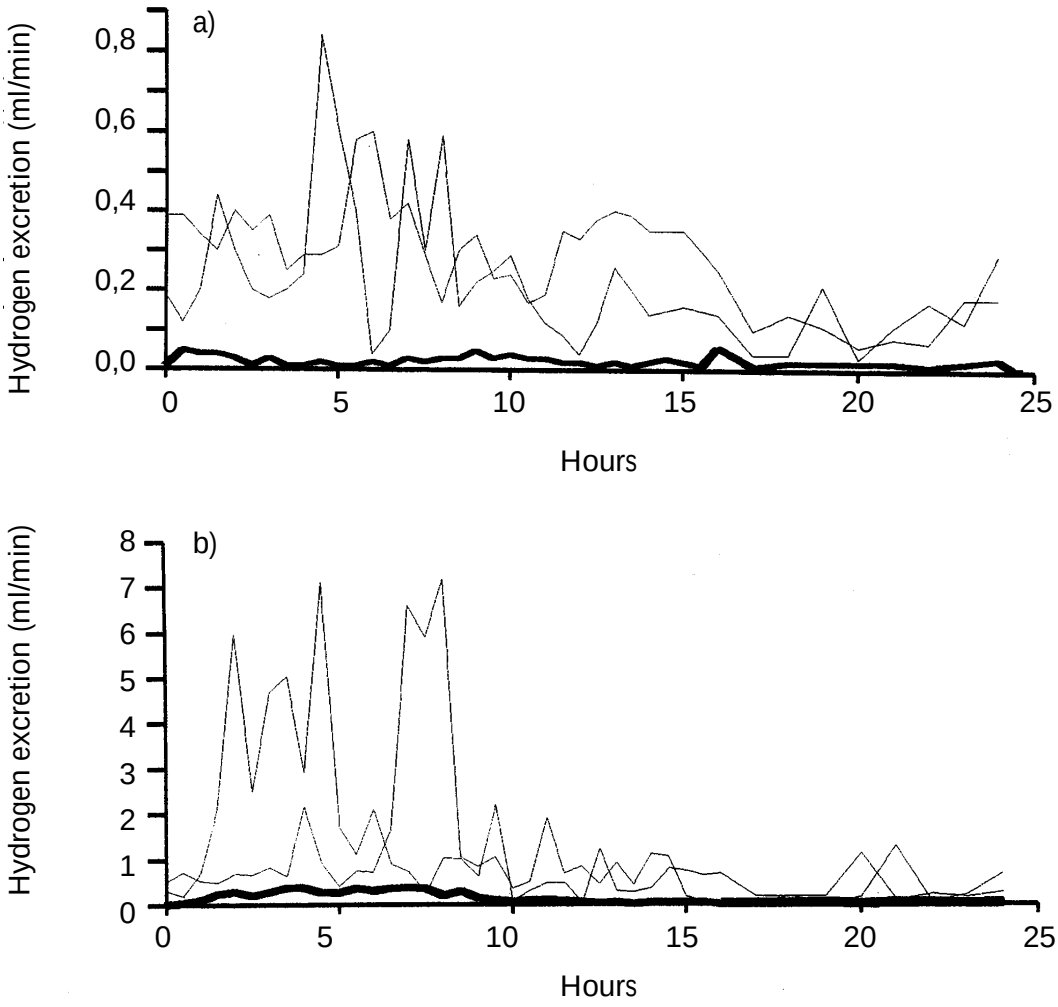

Figure 19 — Total hydrogen excretion rates in ten healthy subjects bold line) compared to two subjects with pneumatosis cystoides intestinalis (datted lines),

a) whilst on a polysaccharide-free diet;

b) after the administration of 15 g of lactulose (from CHRISTL *et al.* [460]).

These studies showed clearly that PCI patients excrete much more H_2 than a healthy population and have inactive H_2 metabolising bacteria in their intestines. The high partial pressure of H_2 in colonic gas is enough to explain the accumulation of gas-filled cysts in the colonic wall [465, 466].

6 – Colitis and pouchitis

6-1 Ulcerative Colitis

The coincidence of the distribution of ulcerative colitis (UC) with the area of the gut most highly populated with bacteria points to a role for intestinal bacteria in its aetiology. However, in no study has a specific species nor mechanism yet been identified. There are no animal models of UC that work in the germ-free state so it would be very surprising if bacteria were not involved in the initiation and maintenance of inflammation in this condition.

ROEDIGER's work in establishing that SCFA, particularly butyrate, were important fuels for the human colonic epithelium led him to examine their metabolism in disease, and in ulcerative colitis in particular. He was the first to show that butyrate oxidation was significantly impaired in UC, both during the active phase of the disease and in remission [467]. This observation has been confirmed by a number other groups [468–471].

These results are summarised in **table XVIII**, and show that butyrate oxidation is substantially reduced even in uninvolved segments of the large bowel in patients with active colitis [468, 470]. One study, in UC patients undergoing

Table XVIII — Butyrate oxidation in ulcerative colitis.

Study	**Tissue**	**Source of specimen**	**Disease activity**	**Effect on butyrate oxidation**
ROEDIGER 1980 [467]	Operative	Descending colon	Quiescent and active	Reduced
IRELAND & JEWELL 1989 [468]	Operative	Descending colon	Quiescent and active	Reduced
CHAPMAN *et al.* 1994 [470]	Biopsy	Ascending and descending colon	Quiescent or mild	Reduced
FINNIE *et al.* 1992 [472]	Biopsy	Ascending and descending colon	Quiescent	No change
WILLIAMS *et al.* 1992 [471]	Biopsy	Rectum	-	Reduced

routine colonoscopy, did not show any difference in butyrate oxidation either regionally in the colon or between UC and controls [472]. In this investigation ileal butyrate metabolism was greater than colonic. A number of methodological differences exist between this latter and the earlier work [473]. ROEDIGER and his colleagues [474] have also shown reduced butyrate oxidation *in vivo*, using a dialysis bag technique and bicarbonate production as a marker, and have gone on to show that, in rats, inhibiting β-oxidation of fatty acids in colonocytes with 2-bromooctanoate produces lesions resembling those of UC [475].

6-1-1 Sulphur Metabolism

If butyrate oxidation is diminished in UC, is this secondary to other cellular events? What is the intra-cellular mechanism of cell damage? A number of lines of evidence point to bacterial sulphur metabolism being involved.

In experimental animals a number of sulphated agents, such as degraded carrageenan, sodium lignosulphonate and sulphated amylopectin, when fed orally in drinking water to guinea pigs and rabbits, induce an acute attack of colitis [476, 477]. The clinical and pathological features closely resemble human UC, except that the lesions extend distally from the caecum. The severity of the colitis is related to the amount of sulphate present in the polymer (**Table XIX**).

Table XIX — Sulphate and colitis in animals.

Polymer	% Sulphate	Experimental colitis
Carrageenan	25 – 30	+ +
Amylopectin sulphate	19	+
Dextran sulphate	19	+ +
Dextran	0	-
Agar	1	-
Chondroitin sulphate	6	-

From M.C.L. PITCHER (unpublished)

Further studies using antibiotics [479–481] or germ-free [482] animals show that normal intestinal bacteria are essential mediators of the disease process. Contrary observations have been described in germ-free rats, but these have been ascribed to species differences in the biological effect of degraded carrageenan [483]. Recently there has been a resurgence in interest in experimental acute and

chronic colitis in mice and hamsters using the dextran-sulphate model, in which the pathology is largely confined to the mucosa of the distal colon [484, 485]. Populations of intestinal anaerobes increased significantly after administration of dextran-sulphate sodium (DSS), an effect nullified with metronidazole pre-treatment with attendent protection from colonic ulceration [486]. It is conceivable that in the carrageenan and DSS models the processing of sulphate, a dietary component, by colonic bacteria yields a product, as yet undefined, that causes immune activation and resultant gastrointestinal damage. This would place UC in the category of immune disorder diseases. An alternative possibility, however, is that a product of bacterial metabolism is directly affecting intracellular metabolism in the colonocyte, leading to a defect in butyrate oxidation. This metabolite could be sulphide, produced by sulphate-reducing bacteria amongst others.

Studies by PITCHER and colleagues [487] in 29 UC patients have shown that, whilst 95% carry SRB in the active phase of the disease, only 55% do so in quiescent disease. The mean $\log_{10}$ counts/g of viable SRB were 3 logs higher in the active disease group than those in remission. Faecal sulphide concentration was higher in those UC patients who were not taking 5-ASA (5-amino salicylic acid).

In the animal models of UC a protective effect of salicylates has been observed. Sulfasalazine reduced the numbers of animals with carrageenan-induced mucosal inflammation by 50%, yet appeared to have no impact on the intestinal microflora [482]. More recently, sulfasalazine and olsalazine have both been found to be effective in preventing deaths in athymic mice, a strain more sensitive to DSS-induced distal colitis than the conventional rat, and a dose-response effect was observed in terms of survival [488, 489]. Clinical response to sulfasalazine treatment has also been demonstrated in DSS-induced colitis in conventional BALB/c mice [490], although two studies of chronic DSS-induced colitis using conventional rats have reported opposite findings with both attenuation and no effect on colonic inflammation with sulfasalazine [491, 492]. Some preliminary results, after sub-group analysis of the most recent data from PITCHER's laboratory, have shown that there may be an effect of salicylates in reducing both sulphate reduction rates and faecal sulphide levels in those patients receiving these drugs [493].

So does SH^-, or do other reduced sulphur compounds, produce the observed metabolic defect in UC cells? ROEDIGER has shown that fatty acid oxidation can be inhibited by a range of S-containing compounds [494, 495]. Of these, the mercapto-acids (2-mercaptoacetate and 3-mercaptopropionate) and sodium sulphite at levels of 1–5 mmol/l selectively diminished fatty acid oxidation by 30–50% without substantially altering glucose oxidation, which reflect the metabolic changes in UC. Selective impairment of butyrate oxidation to CO_2 and

acetoacetate has more recently been demonstrated with sodium sulphide in rat colonocytes at concentrations as low as 0.1–0.5 mmol/l NaHS [496].

The effect of reducing sulphur compounds on butyrate oxidation has also been investigated in healthy human colonic epithelial cells [494]. At a concentration of 2 mmol/l, the order of suppression of fatty acid oxidation at all sites of the colon was hydrogen sulphide > methanethiol > mercaptoacetate, and was most pronounced in the distal colon. Suppression was maximal with 2 mM NaHS which inhibited butyrate oxidation by 75% in the distal colon and 43% in the ascending colon. ROEDIGER concluded that the metabolic effects of anionic sulphide (HS^-) on butyrate oxidation along the length of the colon closely mirror the metabolic and regional abnormalities observed in active UC and may be involved in pathogenesis. Evidence in support of this contention has come from observation of the relationship between luminal sodium sulphide perfused into rat colon and damage to the colonic epithelium [497, 498]. This effect is significant at a sulphide concentration of 0.2 mM—a level which is physiological for human faeces—producing apoptosis, goblet cell loss, superficial mucosal ulceration and impairment of fatty acid oxidation (10–30 mM SH^-).

Preliminary attempts to define the site of sulphide-induced inhibition of butyrate oxidation in the colonocyte have localised a metabolic block at the level of FAD-linked oxidation by butyryl-CoA dehydrogenase [496]. No effect is seen on NAD-linked oxidation which is supported by a recent mRNA analysis for mitochondrial NAD/NADH-dependent dehydrogenases in colonic biopsies from patients with acute UC in which no evidence of enzyme deficiency was found [499].

ROEDIGER's observations have recently been complemented by a study of butyrate uptake in isolated membrane vesicles of the rat distal colon [500]. Intravesicular butyrate uptake was inhibited by 20 mmol/l mercaptopropionate and further increments in concentration produced a dose-response effect, indicating competitive inhibition with the SCFA ion-exchange transporter. Thus, on present evidence, it may be concluded that reducing sulphur compounds are detrimental to colonic metabolism firstly by impairing the transport of n-butyrate into the colonocyte and then by inhibition of n-butyrate oxidation within mitochondria. These mechanisms point to reducing sulphur compounds within the colonic lumen, and hydrogen sulphide in particular, to be candidates for the initiation of ulcerative colitis.

6-1-2 Faecal SCFA in UC

Although ROEDIGER *et al.* [501] reported increased levels of SCFA in faeces of UC patients, in subsequent studies this relationship has not been so clearly demonstrated. VERNIA [502] studied 18 UC patients in whom total SCFA were 87 ± 11 mmol/kg, 20 patients with CROHN's 132 ± 13, and 16 healthy controls 133 ± 12. In a subsequent paper [503] SCFA were found to be high in quiescent and mild UC: 163 ± 64 and 148 ± 63 mmol/kg respectively, but significantly lower in severe disease: 65 ± 47 mmol/kg. Butyrate levels were particularly low in severe UC: 4.3 ± 5.0 mmol/kg compared to controls 13.7 ± 8.4, the inference being that in severe disease there is a relative butyrate deficiency in addition to impaired butyrate oxidation.

However, SCFA levels in faeces are not a particularly sensitive guide to SCFA metabolism in the colon and, in general, the worse the diarrhoea the lower the concentration becomes [191]. Information on production rate would be more useful but is difficult to obtain.

VERNIA's group also noted increasing lactate concentrations in UC which correlated closely with disease severity [503]. High luminal levels of lactate can induce diarrhoea and may lead to mucosal inflammation. Lactate production is favoured during fermentation by high substrate availability and low pH. However, recent studies have suggested that the lactate may be of mucosal origin [504]. It is unwise, therefore, to draw too many conclusions from the study of faecal SCFAs.

6–1–3 SCFA Enemas

The suggestion that diminished butyrate oxidation in UC might be important in the inflammatory process, coupled to observations of low SCFA in the stool of UC patients with severe disease, has prompted a number of groups to try using SCFA to treat UC (**Table XX**).

The first such report [505] was of an open trial using a solution of 80 mmol/l acetate, 30 mmol/l propionate and 40 mmol/l butyrate in twice daily enemas in patients with distal disease. Of the ten patients who completed the 6-week study, nine were improved. BREUER is now (1994) [506] conducting a multicentre randomised placebo-controlled study in the United States Mid-West using the same SCFA mixture versus saline. Preliminary results show 53% of UC patients improved compared to 26% of those on placebo.

The effect of enemas containing butyrate alone (100 mmol/l) has been reported by SCHEPPACH [507]. Ten patients with left-sided UC who were unresponsive to, or intolerant of, standard treatment were given either butyrate or sodium chloride enemas twice daily for two weeks, each in a randomised single

Table XX — SCFA enemas in ulcerative colitis.

Study	Design	*N*	Formula	Outcome
Breuer 1991 [505]	O 6w x2	12	80:30:40	Improved
Scheppach 1992 [507](1)	P 2w x2	10	B 100	Improved
Senagore 1992 [508]	M 6w x2	14	60:30:40	Improved
Vernia 1993 [509]	P 6w x2	20	80:30:40	Improved
Vernia 1995 [510]	P 6w x1	40	80:30:40	Improved
Breuer 1994 [506](1)	P 6w x2	17	80:30:40	Improved
Steinhart 1995 [511]	P 6w x1	19	B 80	No change

O = open trial; P = placebo controlled; M = multiple arms; w = weeks;
Formula = acetate:propionate:butyrate (mmol/l); B = butyrate alone (mmol/l).

(1) Since this table was compiled two abstracts have been published of double-blind placebo controlled (saline) studies in distal ulcerative colitis. In that of Breuer *et al.* (*Gastroenterology* 1996;110:A873) there was clinical and histological improvement which did not reach statistical significance. In the study of Scheppach *et al.* (*Gastroenterology* 1996;110:A1010) there was significant clinical and histological improvement in both treated and placebo groups.

blind cross-over study. After butyrate administration, stool frequency, rectal inflammation and upper crypt labelling index all fell significantly. Early reports of similar studies in progress also look equally promising [509-511], although Steinhart's updated report [512] shows no benefit from a nightly enema of butyrate given for 21 days to 19 UC patients. Many of these were already being treated with steroids and 5-ASA preparations which may have masked any benefits of the enemas.

In the only comparative study of SCFA enemas with conventional treatment, Senagore [508] compared hydrocortisone, 5-ASA and SCFA enemas in 45 patients with distal UC. The SCFA enema comprised acetate, propionate and butyrate and was given twice daily for 6 weeks. All treatments were effective with 12 out of 14 UC patients on SCFA showing a benefit. The lack of potential side effects from SCFA compared with steroid and 5-ASA is clearly a potential advantage.

Thus, it appears that SCFA will prove to be an effective treatment for many UC patients. Where does this leave the sulphur story? If S-compounds inhibit β-oxidation in the way that Roediger suggests, then high concentrations of butyrate might be able to overcome the block, simply by mass action. A direct attack on SRB and attempts to reduce SH^- production in the colon might also be effective

unless there is an inherited disorder of S-metabolism in the colonocyte of UC patients [513]. This might explain why not everyone who carries SRB gets UC. Nevertheless, reduction of SH^- in the colonic lumen should offer an effective therapeutic approach.

6-2 Diversion colitis

In 1981, GLOTZER *et al.* [514] described a condition in which mucosal inflammation occurred in segments of the large intestine from which the faecal stream had been diverted by proximal colostomy. This "diversion colitis" resembled UC in many ways but the inflammation is cured by reanastomosing the bowel and thus allowing the faecal stream back into the defunctioned colon [514, 515]. Various causes have been postulated, including changes in the bacterial flora [516, 517] but the most likely is loss of an essential trophic or nutritional factor from the faecal stream, which is probably SCFA [517]. Diversion colitis parallels a number of other colitides in which SCFA production is reduced, such as occurs in starvation and famine, or utilisation impairment in UC [518].

HARIG *et al.* [519] tested these theories in four patients with diversion colitis. They showed by breath hydrogen testing that no fermentative flora were present in the excluded segment, and neither were there any recognised pathogenic bacteria. Total SCFA concentration in material aspirated from the excluded segments was 0, 4.0 and 0.8 mmol/l for acetic, propionic and butyric acids respectively. They then treated the patients with SCFA enemas comprising 60 mmol/l acetate, 30 mmol/l propionate and 40 mmol/l butyrate, twice daily for 2–6 weeks. All subjects showed clinical improvement and disappearance of histological changes of inflammation. Control periods using either no enemas or saline did not lead to any improvement.

A double-blind study was then undertaken by GUILLEMOT *et al.* [520] in 13 patients who were given the same enema mixture as in HARIG's study, but for two weeks only. No improvement in either endoscopic appearances or histology of the mucosa was observed. It is therefore probable that treatment for longer than two weeks is needed. Further trials are awaited, but at this stage the evidence suggests that in these patients absence of SCFA from the bowel lumen is damaging.

6-3 Pouchitis

Pouchitis is another inflammatory condition of the intestine, this time occurring in the distal ileal loops which are used to fashion an artificial rectal reservoir in patients who have had their large bowel and rectum removed for UC. It is very common after colectomy and is associated with reduced SCFA levels in luminal contents [521, 522], *e.g.* 56 ± 13 (SEM) mmol/l pouchitis versus 139 ± 8 mmol/l healthy pouch (N = 6). SCFA production from faecal homogenates incubated *in vitro* is also reduced, but L-lactate levels are increased three-fold. No bacteriological differences have been observed, and the condition resolves rapidly with metronidazole treatment.

There are sporadic reports of the use of SCFA to treat pouchitis. One group used SCFA enemas in two patients for four weeks without benefit [523] whereas another have used butyrate suppositories [524] also without great benefit. In these same patients, however, glutamine suppositories led to improvement in 60% of patients. Glutamine is the primary fuel for the ileal mucosa, rather than butyrate or other SCFA.

7 – Bowel habit and constipation

7-1 Bowel habit

7-1-1 Frequency

Most adults living on Western style diets pass one stool/day, with 95% of the population going between three times/day and three times/week. The frequency of defaecation is greater in early life at four times/day in the neonatal period (range 1–9), with breast-fed babies having a higher rate than bottle-fed babies. The adult pattern of once a day is reached by the age of four years [525]. As old age (over 70 years) approaches, there is a tendency for bowel habit to become less frequent. In developing countries the modal frequency is nearer twice a day.

7-1-2 Consistency

In the normal population, faecal form and consistency varies from hard, small, fragmented droppings through cylindrically shaped stools to soft porridge-like amorphous material. It has been measured by a variety of means including visual assessment [526], subjective evaluation [527, 528] and by using a penetrometer [529]. Stool consistency is not uniform and may vary within a single motion. In general, as daily stool weight increases so stools become softer and less well formed. Consistency is related to water content, which is normally 70–80%. Men pass softer stools than women. Constipated subjects generally pass harder stools with a water content of less than 70% [527, 530]. It is the hardness and dryness of stools that is thought to cause discomfort on defaecation.

7-1-3 Stool Weight

In the United Kingdom, daily stool weight is about 110 g/d with a range 40–260 g/d. Men pass significantly more than women and the young more than the elderly [13]. **Figure 20** shows the distribution; 47% of men and 51% of women have stool weights less than 100 g/d, and 17% of women and 1% of men pass less than 50 g/d.

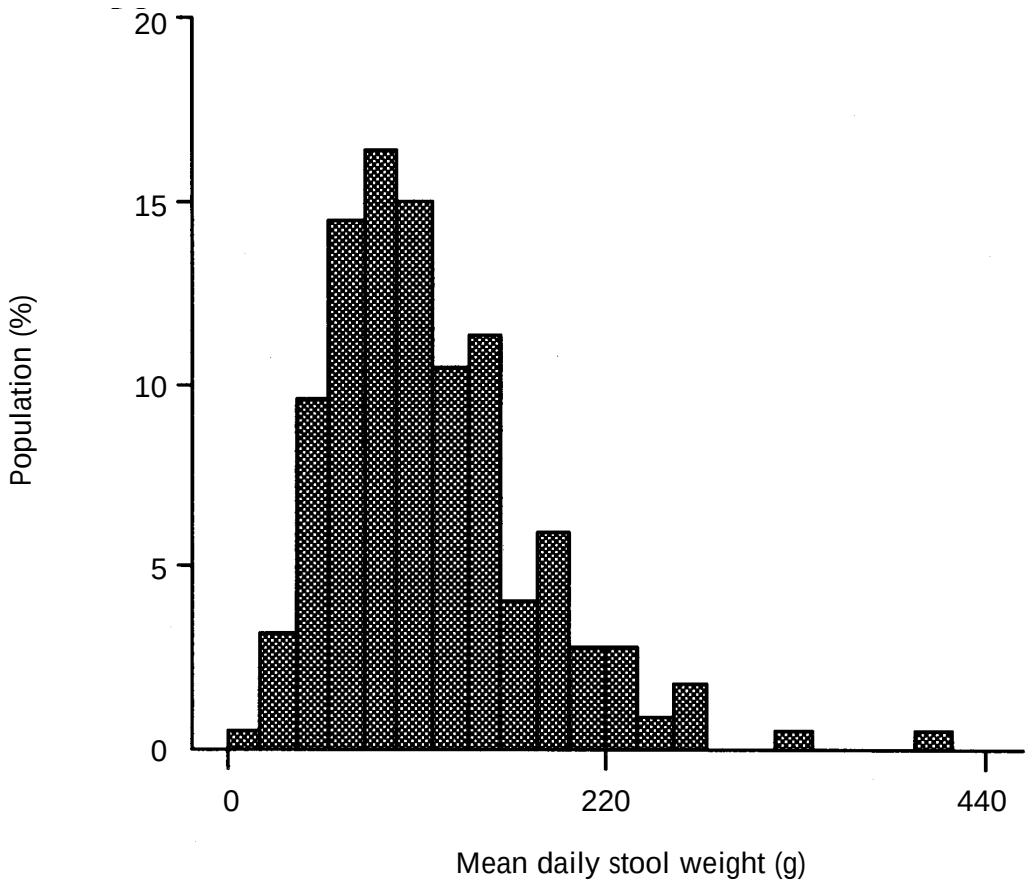

Figure 20 — Frequency distribution of mean daily stool weights in 220 healthy UK adults (from CUMMINGS *et al.* [13]).

Stool output is very variable from day to day in individuals [526, 529, 531, 532]. This means that to measure daily stool weight accurately requires complete faecal collections for at least five consecutive days. The variation is the result of hormonal cycles, such as the menstrual cycle, dietary patterns, mood changes, social pressures, *e.g.* whether defaecation has to be suppressed or not, travel and, possibly in hot climates, fluid intake.

Changes in bowel habit are reported in long-distance runners. However, moderate exercise sufficient to improve physical fitness, but with diet held constant does not affect stool weight or transit time [533].

Bowel habits vary substantially in world populations (**Table XXI**), the range in mean stool weights being 72–470 g/d. The United Kingdom is notably towards the lower end of the range, along with the United States and New Zealand, while rural populations in developing countries pass more than double the amount of the United Kingdom.

Table XXI — Stool weight in various population groups

Country	**Stool weight** (g/d)	**Number of subjects**
UK: Scotland (F)	72	43
UK: Scotland (M)	93	47
USA: New York (M&F)	99	99
New Zealand: European (F)	113	25
New Zealand: Maori (F)	119	19
USA: Hawaii - White (M)	120	18
USA: Hawaii - Japanese (M)	120	47
Japan: Hyogo (M)	133	11
UK: England & Wales (F)	134	41
Denmark: Copenhagen (M)	136	30
UK: England & Wales (M)	145	59
Sweden: Malmo, Umea (M&F)	150	45
Denmark: Taarnby (M)	151	60
Denmark: Them (M)	169	30
Malaysia: Indian Urban (M&F)	170	
Finland: Helsinki (M)	176	30
Japan: Akita (M)	195	17
Finland: Parikkala (M)	196	30
Finland: Kuopio (M)	209	75
Malaysia: Chinese Urban	227	
India: New Delhi	311	514
Peru (M&F)	325	20
Malaysia: Malay	465	
Uganda	470	15

Modified from CUMMINGS *et al.* [13]

7-1-4 Transit time

There are two principal factors controlling human bowel habit, namely diet and transit time. Transit, which is the time it takes material to pass from the mouth through the gut to the rectum, is largely genetically determined as far as is currently known. It is probably controlled by those factors which affect bowel habit such as hormonal status, stress and social factors [208, 534].

A variety of methods for measuring transit time have been described, but all are essentially variations on two themes. Transit time may be measured by giving a single dose of a marker substance, usually radio-opaque pellets, collecting faeces until all are recovered, and calculating the mean time the markers have taken to traverse the gut. Alternatively, markers may be given with every meal, and faeces collected until a steady state is reached (where input is approximately equal to output of markers), when the number of markers retained in the gut is a measure of transit time (number of markers retained divided by marker input rate equals transit time) [535, 536].

To avoid collecting faeces, abdominal radiographs can be taken to assess marker retention and distribution. This has the advantage of simplicity and allows transit time in the different segments of the colon to be determined [15, 537–541], but radiation exposure limits the usefulness of this technique.

Normal Values

In the United Kingdom, mean transit time (MTT) is 70 h but, as **figure 21** shows, the distribution is very skewed and median MTT is 60 h, with men 55 h and women 72 h. The range (95%) is 30–168 h. Transit time in other countries is faster, particularly in Africa, where 24–48 h is the norm [14]. Differences in methods make comparisons difficult.

Transit time varies greatly from day to day and from week to week in individuals. Such variation is presumably due to the same factors that influence bowel habit, though much is still unknown about this important parameter of colonic function.

MTT and stool weight are closely related [14, 542–544]. At transit rates of 80 h or more stool weight is low but increases as transit speeds up to 40–50 h, after which changes in transit time are not associated with particularly great changes in stool weight. Transit determines stool weight, not *vice versa*. Slowing MTT with drugs decreases stool weight [203], but diet shows a less consistent effect [545].

Transit times through different regions of the colon can be measured using oral doses of radio-opaque markers and taking abdominal x-rays at intervals. Reported times are 7–24 h for the right colon, 9–30 h for the left colon and 12–44 h for the sigmoid colon and rectum [15, 540, 542].

The time that material takes to pass through the colon is important, not only as a standard against which to judge constipated patients but also because time is a determinant of bacterial activity [203].

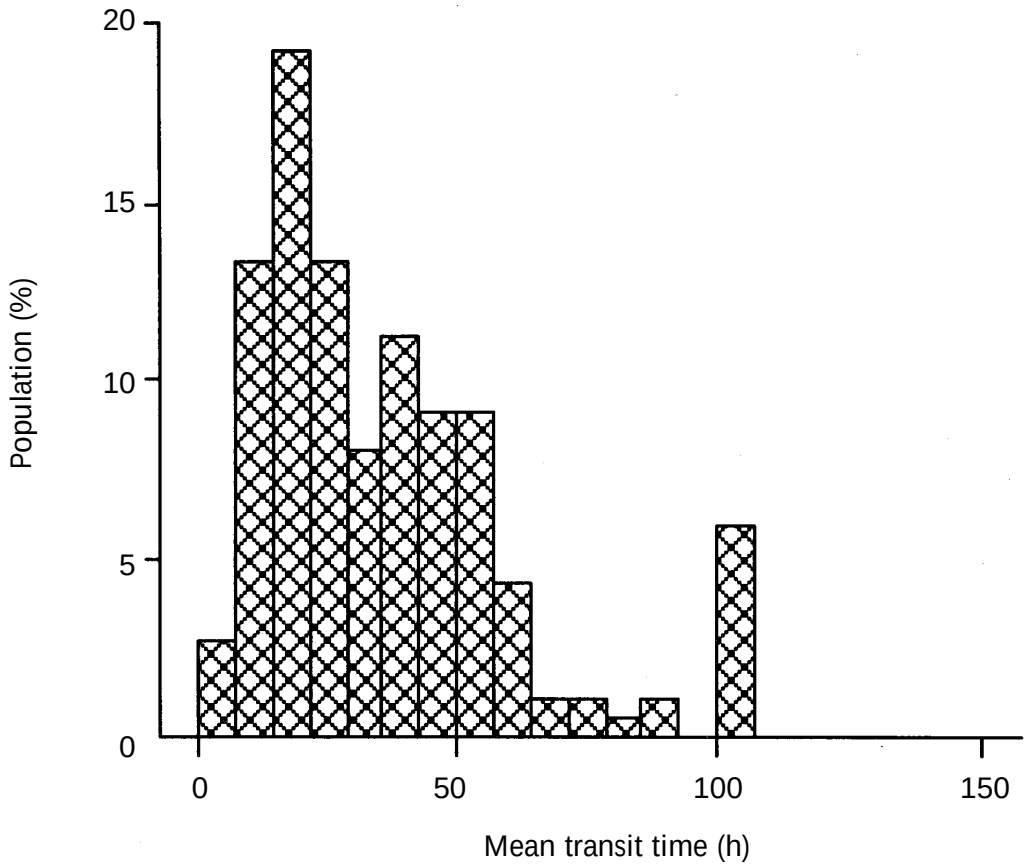

Figure 21 — Frequency distribution of mean transit times from 187 healthy UK adults. The final column of the histogram represents transit times greater than 168 h (from CUMMINGS *et al.* [13]).

7-1-5 Diet and Bowel Habit

Dietary fibre, specifically NSP, is the only dietary component to have been shown consistently and over many generations to control bowel habit. Feeding major components of the diet such as fat and meat has no effect on bowel habit [546, 547]. Neither—in moderate amounts—does sulphate, which is a component of well-known laxatives, affect bowel habit [447].

Figure 22 gives the combined results of eleven published studies in which dietary NSP intake has been measured carefully and accurate stool collections made, whilst other dietary components were kept constant. A total of 26 separate dietary periods are included in which 206 persons took part. These data show clearly the effect of increasing NSP intake on stool weight. The regression (stool

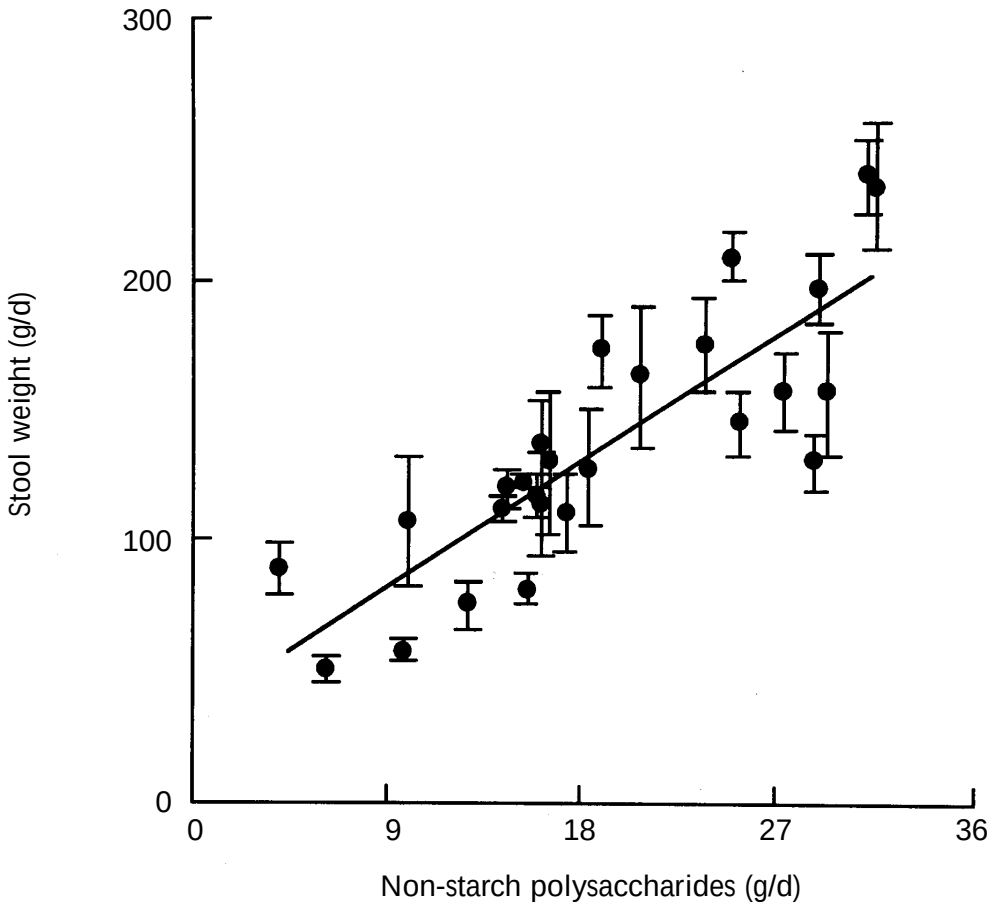

Figure 22 — Mean daily stool weight (±SEM) in 11 groups of healthy subjects (total *N* = 206) eating controlled diets (26 dietary periods) with different amounts of NSP (from CUMMINGS *et al.* [13]).

weight = 5.3 NSP intake + 38) predicts that at an NSP intake of 12.5 g/d, which is the UK national average (MAFF, 1990) [548], stool weight is 104 g/d (95% CI, 99–108 g/d) and that on an NSP-free diet it would be 38 g/d. Median stool weight in the UK is 106 g/d so these controlled experimental studies of diet and bowel habit allow us to predict the relationship between stool weight and NSP intake for a population. The figure includes data from many different sources of NSP. If bran and purified NSP are excluded, predicted stool weights are slightly lower (97 g/d at 12.5 g NSP intake).

All sources of NSP will produce an increase in stool weight if tested under sufficiently rigorous conditions. However, the change in stool output varies amongst the different types. How do they compare with one another?

Many hundreds of papers have been published on this subject and the key ones are summarised in **figure 23**. This is a compilation of faecal weight data from around 120 papers published between 1932 and 1992 detailing 150 separate studies [549, 550]. It shows the average increase in stool output expressed as grams of stool (wet weight) per gram of "fibre" fed. Studies were included only if they contained quantitative data on changes in stool weight and had at least one control and one test period.

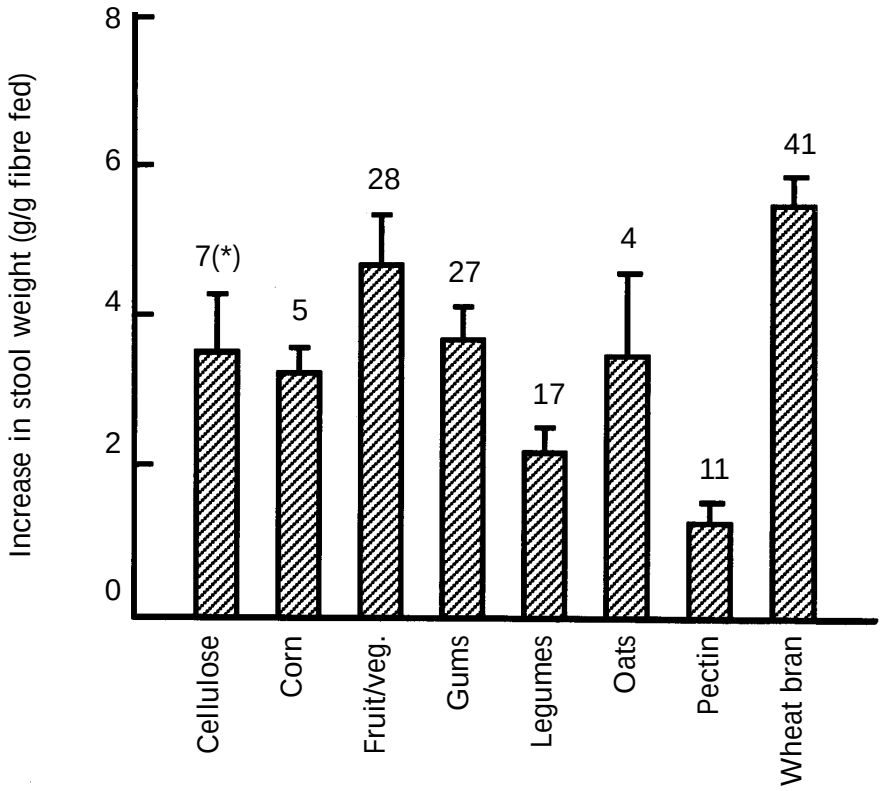

Figure 23 — Average increase in wet stool weight per gram of "dietary fibre" fed from various sources. Results are from 140 published studies; (*) = number of studies used to calculate average and SEM (from CUMMINGS [549, 550]).

Wheat as a source comes out as the most effective at 5.4 (0.7 SEM) g stool/g fibre fed as averaged from 41 studies. Raw bran at 7.2 g/g is more effective than cooked bran, 4.9 g/g ($p < .05$), but has the disadvantage of containing 3% phytate, a known inhibitor of the absorption of divalent cations (calcium, magnesium, zinc and iron). Fruit and vegetables are remarkably effective, 4.7 (0.7) g/g, and with wheat are well ahead of the rest. Whilst many people believe fruit and vegetables to contain mainly soluble NSP, in fact this is not the case. They contain significant

amounts of insoluble NSP and, moreover, most of these laxative studies were done using whole foods containing intact plant cell walls, which probably contributes to their effect. After fruit and vegetables come gums and mucilages at 3.7 (0.5) g/g. This includes most of the bulk laxatives. The league table then progresses through cellulose 3.5 (0.8), oats 3.4 (1.1), corn 3.3 (0.3), legumes (mainly soya) 2.2 (0.3) and lastly pectin 1.2 (0.3).

How reliable are these rankings? Within each group there is great variability although, despite this, the overall differences are statistically significant (by ANOVAR F 4.78; $p < .001$). The variability is due in part to the inherent difference in individual responses and to varying experimental designs, some of which are uncontrolled diets. A further problem is lack of consistency in methodology for measurement of dietary fibre. Around 20 different methods were used in these studies, some of which give only a rough approximation of the true NSP content of the food or source. These laxative properties of NSP are used in the prevention and treatment of constipation.

Mode of Action of NSP

Although a lot of progress has been made in understanding how NSPs exert their laxative effects it remains difficult to predict for any given NSP source. The reason is largely because there is a wide variety of NSP types and their fate in the gut can vary substantially.

Figure 24 summarises the major effector pathways for NSP in the colon. There are three principal contributory mechanisms to the final increase in stool bulk: mechanical, bacterial and motor [545, 549].

– Mechanical properties

The mechanical effect has been known longest and in its simplest form equates the presence of undigested plant cell wall structures in the colon with increased bulk of contents through an ability to hold water within their cellular structure. This is a physical property of NSP and one which distinguishes it from almost every other dietary component. Measuring physical properties and thus being able to predict the laxative action of NSP has proved to be very difficult, although EASTWOOD and colleagues [139, 551] have made a number of attempts to do this. Nevertheless, there is still no simple way of characterising NSP in such a way that can be applied to food and purified sources to allow their overall action to be foretold. This may be because the range of physical types encountered in the plant cell wall is great and includes everything from rigid, lignified structures in wheat bran pericarp to the weak gels of growing plant shoots. Nevertheless, simple physical bulk in the colon does lead to greater stool weights [552].

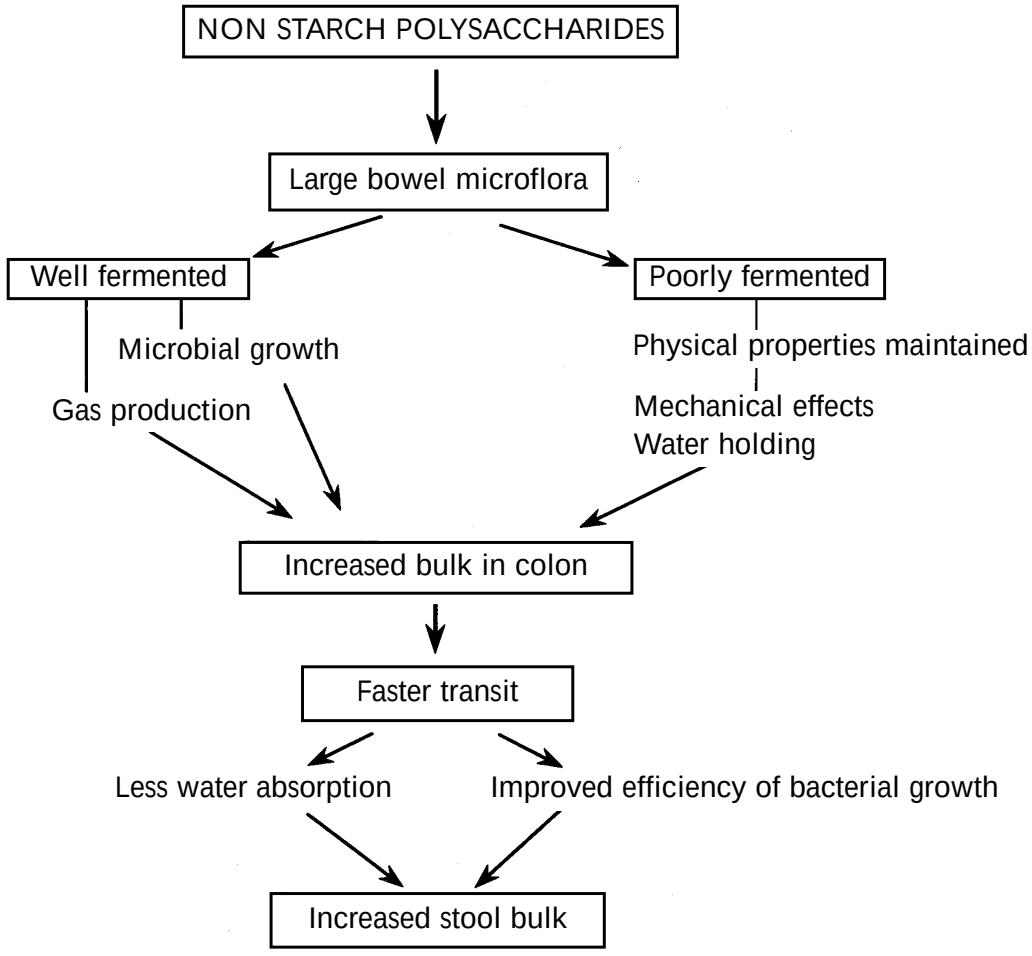

Figure 24 — Mechanisms by which dietary unabsorbed carbohydrate such as NSPs, resistant starch, oligosaccharides, etc., may increase stool bulk (from CUMMINGS [549]).

– Bacteria

An important new dimension to our understanding of the mode of action of NSP came when it was shown that much of it is fermented in the colon and stimulates bacterial growth [22]. The increased stool bulk seen with many sources of NSP largely comprises bacterial cells (biomass) which have grown using the carbohydrate as an energy source. Bacteria are 80% water and so an effective faecal bulker. However, the conversion of NSP to biomass is only about 30% efficient in man [553] (*i.e.* biomass = about 0.3 original NSP in g) so NSP which is not broken down is a better bulking agent in general provided it has the appropriate physical properties. When antibiotics are given to suppress

fermentation, and presumably bacterial growth, faecal NSP excretion (measured as neutral detergent fibre) rises and so does faecal bulk [554].

Many sources of NSP are only partly fermented, and others are fermented at different rates. Attempts to relate this to faecal bulking properties have been made with some success. Rapidly fermented NSPs, such as pectin and guar, seem to be poor laxative agents, whilst more slowly and incompletely fermented sources, such as ispaghula, are better [551, 555–557]. In general it is accepted that insoluble NSP, usually from grains and seeds, is better at faecal bulking than soluble NSP from other sources.

Bacterial fermentation also produces gas which may contribute to faecal bulk.

– Motor effects

The third important controlling factor in the faecal bulking effect of NSP in man is motor activity or transit. Increased bulk of gut contents stimulates faster transit, which in turn will reduce the desiccation of stool. Equally importantly, faster transit improves the yield of biomass from carbohydrate because of reduced requirements for maintenance energy by bacteria [558, 559]. Thus faster transit leads to greater stool weight—a relationship established in many studies [203].

Dangers of NSP

The dangers of eating fibre are few. Because fibre is fermented in the colon, gas is produced and therefore patients who increase their fibre intake too quickly will experience abdominal distension, bloating, pain and increased flatus. A slow build-up in dose allows the bacteria and gut to adjust. Other complications of fibre therapy, such as intestinal obstruction and mineral malabsorption, are largely theoretical. However, because of the phytic acid content of raw bran and All Bran, these products should be used with caution in patients whose mineral balance may be precarious such as those who are young, elderly, or pregnant [560]. High fibre diets (and bulk laxatives) are not suitable for the treatment of constipation due to neurological disorders or obstructive lesions of the gut and are ineffective in the more severe forms of constipation associated with disordered defaecatory reflexes, very slow transit and where there is immobility and faecal impaction in the elderly.

7-2 Constipation

Constipation is a disorder of motor activity of the large bowel. For more detailed information, any of a number of recent reviews should be consulted [6, 561–563]. Although traditionally defined in terms of bowel regularity, stool consistency and weight, in practice the main symptom in constipation is straining on defaecation. In addition, abdominal discomfort, distension and incomplete rectal emptying are all considered part of the condition [528, 564, 565]. Constipation may present as a very infrequent bowel habit, some patients passing a stool only weekly or even monthly. When people are questioned about their bowel habit, however, a number will complain of constipation but nevertheless pass a stool daily [528, 564, 566]. Stool frequency is thus not an infallible guide to the diagnosis of constipation. Clinically, a change in bowel habit is equally as important as defining defaecation pattern frequency.

Despite the belief that constipation is characterised by low stool weight (among other criteria), stool collections are seldom made in constipated subjects. In a meta analysis of data from eight groups of subjects who complained of constipation, mean daily stool weight for the whole series (n = 209) is 48.6 g/d [549]. As some of these subjects, chosen from various clinical trials, probably represent the more extreme end of the spectrum of constipation, the true mean may be higher. Thus, stool weight in constipation is low, but the range overlaps substantially with what is generally regarded as normal. It is not surprising, therefore, that constipation is a common complaint in many countries.

Total gut transit time is prolonged in many patients with constipation [530, 567]. Absolute values are not readily available, however, because the many techniques using radio-opaque pellets adopt widely differing criteria for arriving at a transit time value. Thus, the dividing point between normal and constipated whole gut transit times, reported as between 67 and 120 h, is dependent on the method of measurement and the population studied [540, 568, 569]. Another difficulty in setting a dividing line between normality and constipation is that many constipated patients have transit times well within the normal range, whatever method is chosen [570, 571]; a safe cut-off point is probably 120 h.

Studies of transit through the major regions of the large intestine have proved useful in distinguishing different types of constipation. Several patterns have been described, including generalised slowing throughout the whole colon (colonic inertia, slow transit constipation) seen in young women and in response to drugs or systemic disease [572]. Right-sided slowing is most often seen in ulcerative colitis [573] and in some lesions of the central nervous system [574]. Delay on the left side or in the rectosigmoid region is associated with disorders of defaecation and has been called “outlet obstruction” [571, 575, 576].

7-2--1 Constipation and Diet

An inappropriate diet is one of the major causes of constipation and diet is the first therapy to initiate in new cases which do not have another treatable cause. Diets which lead to constipation include: those characteristic of Western culture (*i.e.* low NSP), diets in old age where food intake is reduced (and activity also), therapeutic diets such as those for slimmers, gluten-free diets, traditional diabetic diets, and "low residue" diets, the diets of those suffering from anorexia nervosa and bulimia, diets eaten during travel and on holiday, and enteral/parenteral feeding regimes.

NSPs are the major dietary component to affect bowel habit, so how much is required to treat constipation? Intakes in the UK are around 12–13 g/d [577–582], although in some populations [583] intakes are higher, as they are in vegetarians.

In constipation there is no universal dose of NSP. The amount required is that which will produce a satisfactory bowel habit, usually one soft stool daily, or on alternate days. The aim should be to increase the patient's intake of NSP up to 24 g/d. Those who fail to respond to larger doses should be looked at carefully for organic causes of constipation. Simple dietary constipation usually responds to quite modest increases in fibre intake.

Dietary NSP intake can be increased by:

- increasing bread intake to about 200 g (six slices) per day and changing to 100% wholemeal;
- eating a wholewheat or rye based breakfast cereal such as Shredded Wheat, Weetabix, rye or bran flakes;
- increasing fruit and vegetable intake;
- eating more legumes such as peas, beans and lentils;
- if necessary, an occasional dose of a concentrated fibre food such as bran and All Bran, although there are some disadvantages here.

7-2-2 Bulk Laxatives

In addition to diet, bulk laxatives are a useful adjunct to the treatment of constipation. Bulk laxatives are very concentrated sources of NSP but how good are they at altering bowel habit?

Three out of the four most commonly used sources are not plant cell wall material. Ispaghula and sterculia are two chemically contrasting polysaccharides. Ispaghula is a seed mucilage from the Plantago family and is mainly a water soluble arabinoxylan [551, 555, 584, 585]. Sterculia, a plant gum, is also water

soluble but comprises largely galactose, mannose and uronic acids [177, 586, 587]. Their fate in the gut is probably to be partly degraded, but also to retain some gel-forming potential right through into faeces. Methylcellulose probably resists digestion completely. However, there are very few studies where faecal recovery of these materials has been determined. In fact the paper by PRYNNE and SOUTHGATE (1979) [555] reports probably the only study in man using controlled diets. We are thus relying on results from four individuals plus some *in vitro* and animal fermentation experiments, in conjunction with the chemistry, to interpret how these laxatives work. Some fairly straightforward feeding trials could yield valuable results.

Figure 25 summarises the results of about 40 published studies in which it is possible to obtain a quantitative estimate of the change in stool weight attributable to varying bulk laxative preparations other than bran.

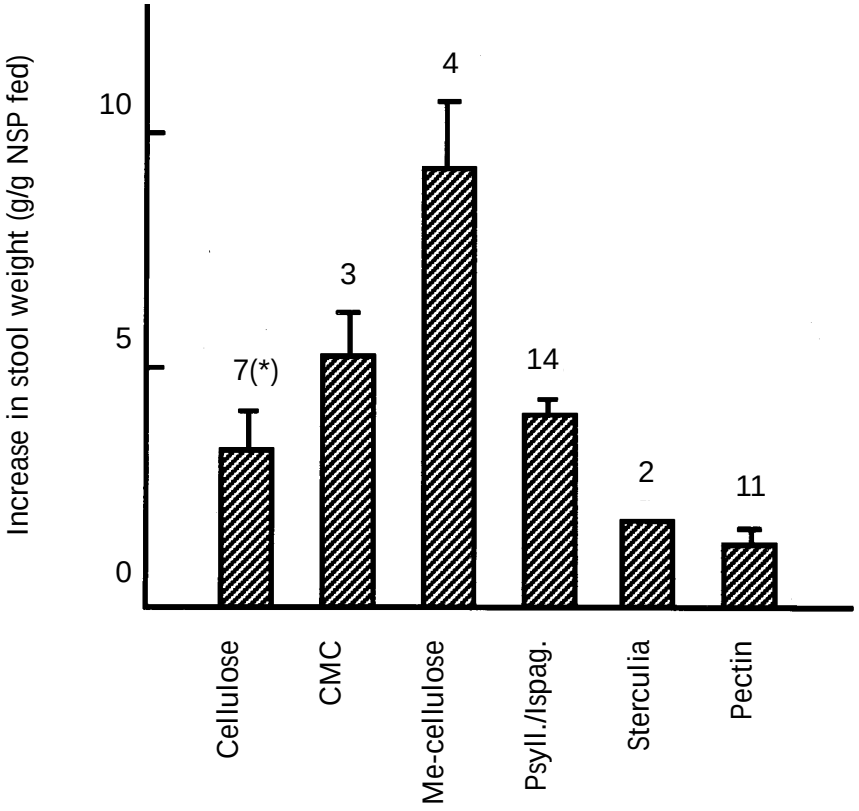

Figure 25 — Average increase in wet stool weight per gram of NSP from various bulk laxatives, cellulose and pectin. Results are from 41 published studies ; (*) = number of studies used to calculate average and SEM; CMC = carboxy-methylcellulose (from CUMMINGS [549]).

Psyllium/ispaghula has been the most studied and is an effective stool bulking agent producing an average 4.0 (0.4) g wet weight stool/g ispaghula NSP fed (14 published studies involving 117 subjects). This is a similar effect to plain cellulose which gives 3.3 (0.8) g/g (seven studies and 44 subjects). Cellulose derivatives are much more effective with carboxymethylcellulose at 5.3 (0.9) g/g and methylcellulose at 9.3 (1.4) g/g. However, the number of studies is small although one might expect methyl substitution to increase the water-holding capacity of cellulose and thus perhaps increase its faecal bulking ability, provided it is not digested. Sterculia is much less effective as a laxative but again the number of studies here is too small to be certain about this. Some straight within-subject comparisons would be useful.

Bran

A great deal is known about the properties of bran—-historically the original laxative. **Figure 23** shows that there are 41 published studies which combine together to give an average increase in stool weight of 5.4 g/g bran NSP fed. Bran NSP is known to be poorly fermented, retain its cellular structure, hold water and speed up intestinal transit. Some stimulation of biomass results [22]. Raw bran is more effective than cooked bran [532] but contains more active phytate; coarse bran is better than fine bran [144, 550, 588].

It is worth emphasising that there are many sources of bran available and not all have equal amounts of NSP. **Table XXII** compares Trifyba (available on prescription in the UK) with a standard wheat bran and Kellogg's All Bran. Trifyba

Table XXII — Composition of brans (% as eaten).

Composition	Trifyba	Wheat bran	Kellogg's All Bran
NSP (dietary fibre)	69	36	24
Starch	0	23	28
Sugars	0	4	19
Protein	7	14	14
Fat	5	5	3
Phytate	<0.02	3.0	3.2
Salt (NaCl)	0.02	0.2	2.3
Moisture	4	8	3

See [549] for data sources.

is a useful addition to the formulary because it has a high NSP content (three times that of Kellogg's bran) and is virtually phytate free. All Bran contains nearly 20% sugars and is one of the highest sources of phytate in the diet. Presently there are few published studies of the efficacy of Trifyba. Some direct comparisons with the other bulk laxatives would be useful.

7-2-3 Other Laxative Dietary Constituents

Finally it is worth noting that NSPs are not the only part of the diet to have laxative properties.

There are of course specific laxative substances present in foods such as prunes (diphenylisatin) and sulphate ion is cathartic if taken in sufficient amounts. However, the diet also contains other carbohydrates which escape digestion in the small bowel, and are fermented in the colon. The principal types are resistant starch, which is laxative in man (see chapter 3) and oligosaccharides such as fructooligosaccharides found in onions, leeks, garlic and chicory [52], polydextrose and of course the non-absorbable disaccharides lactulose and lactitol.

8 – Diet and Large Bowel Cancer

8-1 Introduction

Large bowel cancer is one of the commonest causes of cancer death in Western countries. In areas where previously there was a low incidence, such as Japan, rates are beginning to rise as the traditional Japanese diet changes towards a more Western type. Large bowel cancer is, above all other cancers, the one for which there is most evidence of diet being involved in its cause and also in its prevention. Furthermore, the genetic abnormalities associated with these tumours are better defined than for many of the other common cancers and thus it offers one of the greatest hopes for a public health prevention strategy through dietary modification.

In England and Wales in 1992, colorectal cancer was responsible for 11.6% of all cancer deaths (8,749) in men and 12.6% in women (8,654). In men it was the second commonest cause of cancer death after lung and third commonest after breast and lung in women (**Table XXIII**).

In many European countries the data are similar (see IARC Cancer Incidence in Five Continents 1992) [590]. For example, in Maastricht—a region close to the borders of Belgium—between 1986 and 1988, colorectal cancer was second commonest in both men and women, being 13.4% and 16.4% of incidence respectively (**Table XXIV**). Across Europe the major causes of cancer death are lung, colorectal, breast, prostate and stomach, with lymphoma, bladder, uterus, ovary, pancreas and kidney somewhat further behind. Three out of five of these major cancers are related to diet. Outside Europe, the pattern is very similar in North America but in countries such as China oesophageal cancer is common whilst in Africa cancer of the liver and cervix, together with the oesophagus, are more prevalent.

In England and Wales between 1979 and 1992 total cancer deaths from prostate rose very substantially, breast modestly and colorectal slightly, whilst gastric cancer declined. Gastric cancer rates are declining all over the world in conjunction, it is felt, with reduced salt intake and intakes of preserved foods. All this is largely due to better refrigeration and means of transporting and storing foods.

Trends in cancer incidence in Europe are shown in **table XXV**.

Table XXIII — Cancer deaths – England and Wales 1992.

Type of cancer	Deaths	% all cancers
Women		
Breast	13,663	19.9
Lung	10,994	16.0
Colon and rectum	8,654	12.6
Blood and lymphatic	4,694	6.8
Ovary	3,880	5.6
Stomach	3,291	4.9
Pancreas	3,108	4.5
Oesophagus	2,113	3.1
Cervix	1,647	2.4
Men		
Lung	22,668	30.0
Colon and rectum	8,749	11.6
Prostate	8,735	11.6
Blood and lymphatic	5,246	6.9
Stomach	4,994	6.6
Bladder	3,482	4.6
Oesophagus	3,296	4.4
Pancreas	2,926	3.9

Data from OPCS Monitor DH2 93/2. Deaths by cause [589].

Table XXIV — Cancer incidence – Maastricht 1986–1988.

Men		Women	
Type of cancer	**% all cancers**	**Type of cancer**	**% all cancers**
Lung	31.1	Breast	32.4
Colon and rectum	13.4	Colon and rectum	16.0
Prostate	11.3	Blood and lymphatic	6.2
Blood and lymphatic	6.5	Uterus	5.6
Stomach	6.1	Lung	4.4
Bladder	4.1	Ovary	4.3
Kidney	3.6	Stomach	4.0
Pancreas	2.2	Cervix	3.3
		Pancreas	2.5
		Bladder	2.4

Data from IARC/WHO. Cancer Incidence in Five Continents [590].

Table XXV — Cancer trends – Europe.

Decreasing	Steady	Increasing
Stomach Lung - men	Colorectal Ovary Uterus Leukaemia (Breast)	Prostate Oesophagus Lung - women Lymphoma Bladder/kidney Skin

Evidence for an environmental cause for cancer can be gathered from a number of sources such as:

– Worldwide and regional variation in rates
– Time trends
– Migrant studies
– Smoking and alcohol associations
– Other environmental agents
– Diet
– Genetics.

Diet in general may be related to cancer in a number of ways, and these are listed below:

– Source of naturally occurring carcinogens
– Moulds formed on storage
– Products of cooking
– Dietary deficiencies
– Obesity / Total energy intake
– Macronutrient imbalance?
– Protective factors.

8-2 Epidemiology

The epidemiology of large bowel cancer, and particularly the diet associations, have been summarised in a number of recent reviews [591–594]. In epidemiological studies colon and rectum are sometimes separated and on other occasions reviewed together. There is uncertainty as to whether the two

anatomical locations have different etiologies. It is likely they share a number of common causes but there seem to be differences in the patterns of distribution of colon and rectum amongst populations and also between the sexes. For example, rectosigmoid tumours are commoner in Western industrialised nations whereas in countries with low rates of colorectal cancer right sided colonic lesions tend to be more common. There is also the suggestion that rectal cancer is more strongly associated with alcohol consumption, particularly beer drinking [595, 596].

The principal dietary associations with large bowel cancer are fat and meat, which increase risk, and with cereal foods and fruit and vegetables, which reduce risk. The results of case control studies, of which more than 50 have been published for colorectal cancer, are less consistent, possibly because of the methodological problems with these studies. The majority, however, show an increased risk for meat consumption with possibly a particular assocation with red meat and not with fish and seafood. Dairy products and eggs show no clear risk but vegetables and fruit are consistently protective. With regard to dietary fibre, intakes tend to be lower in populations with increased risk of bowel cancer but fibre intake data are notoriously unreliable and poorly obtained [597]. If starch intakes were to be taken into account in addition [199] then the picture might become much clearer. In BINGHAM's (1990) [597] review of over 30 case control studies looking at dietary fibre intakes and colorectal cancer, there was a protective effect although this was most especially associated with fruit and vegetable consumption. An increasingly observed association with colorectal cancer is that of energy intake and body weight. Increased energy and overweight seem to be associated with increased risk. Conversely, studies of physical activity suggest it may be protective [598–600].

8-3 Genetics

The publication in 1986 by HERRERA *et al.* [601] of a case report in which a deletion in the long arm of chromosome 5 was associated with multiple intestinal polyps, mental abnormality and large bowel cancer, led to an intensive search for genetic abnormalities in this tumour. Since then a number of exciting discoveries have been made, particularly by BODMER *et al.* [602] and VOGELSTEIN's group [603–605], such that there are now a number of well established chromosome abnormalities found in this condition (**Table XXVI**). The role of many of these genes is becoming clear and, not surprisingly, they are mostly involved with the control of cell growth, differentiation and DNA repair. Perhaps, equally importantly, what has emerged from the genetic studies is that a single gene defect does not lead to large bowel cancer although the 5q21 abnormality found in familial adenomatous

polyposis (FAP) seems to be particularly associated with this hereditary form of colonic polyps. A number of gene defects are collectively needed to produce the varying stages in the cycle from a normal epithelium through polyp formation, tumour growth and metastasis and it is likely that these have to originate from a single clone of stem cells [606, 607].

Table XXVI — Genetic defects associated with colorectal cancer.

Location	2, 3, 7	5q	12	17p	18q
Genes	HNPCC	MCC APC	k-ras	p53	DCC
% Tumours with mutations	95	> 70	50	> 70	> 70

From [509, 602–605, 608–610].

8-4 Mechanisms

Carcinogenesis is a multi-stage process, the initiation of which is almost certainly due to DNA damage. Possible DNA damaging agents in the large intestine include heterocyclic amines, nitrosamines, free radicals, fecapetaene and possibly other substances. The more commonly found adducts are 06-methyl-guanine and 8-hydroxy guanine. DNA damage alone, however, is clearly not enough to lead to a chromosomal abnormality because there are active DNA repair mechanisms going on in the cell all the time. As yet not much attention has been paid to these DNA repair mechanisms and it is possible that diet may play an important part here. The role of fresh fruit and vegetables could, of course, be preventing free radical damage through the various antioxidant micronutrients present in these foods.

8-4-1 Fat

In the 1970s fat was considered to be the principal risk factor for large bowel cancer and the mechanism, first proposed by HILL and colleagues [611, 612] was through an effect on bile acid metabolism. It had been known for many years that some bile acids were carcinogens in mouse models, and degraded bile acids—particularly deoxycholic acid—were shown in epidemiological studies to be related to risk of colon cancer [612]. Subsequent studies, however, suggested that the epidemiological findings were not so clear and a DNA damaging role for

bile acid has never been established. Recently, however, the theory has received new impetus with the observation that bile acids may stimulate cell proliferation in the colon through the action of diacylglycerol, which activates protein kinase C [613]. In physiological studies, dietary fat increases colonic fat and bile acid levels and the effect can be ameloriated by dietary calcium supplements [614, 615]. In animal studies, fat acts very clearly as a promoter of large bowel cancer and cell proliferation. Fat may also be a precursor of one of the first observed colon carcinogens, the fecapentaenes. Reduction of dietary fat, therefore, still remains a strong component of the preventive strategy for bowel cancer prevention in Western countries.

8-4-2 Meat

Although an association between meat consumption and colorectal cancer has been known since the seminal work of ARMSTRONG and DOLL in the 1970s [616], a really good mechanism has only recently been identified. It is now clear that the cooking of meat leads to the chemical synthesis of heterocyclic amines, of which PhIP has attracted much recent attention [617–619]. PhIP is strongly mutagenic in the classical *in vitro* assays and is found in much higher concentrations than the earlier heterocyclic amines such as IQ and MeIQ which were first observed to be carcinogens in mouse by SUGIMURA *et al.* [619]. However, WATANABE and OHTA (1993) [620], using mutational analysis, have shown that the principal mutations in colorectal cancer cells are base substitutions whereas heterocyclic amines tend to produce frame shift mutations. Recently, BINGHAM and colleagues have shown that increasing meat intake can lead to increased amounts of nitrosamine in faeces in humans [621]. Nitrosamines characteristically produce point mutations in DNA and therefore are probably the strongest current candidate mutagens for large bowel cancer.

8-4-3 Protective Mechanisms

There are a number of ways that fruit, vegetables and cereals may protect against large bowel cancer. The antioxidant micronutrients present in fruit and vegetables may prevent free radical damage to DNA or, as has more recently been suggested, affect the capacity of carcinogen metabolising enzymes in epithelial cells. KADLUBAR *et al.* (1992) [622] showed that some people acetylate or oxidize carcinogens at different rates and that this relates to the activities of key enzymes within the epithelial cell. These genetic polymorphisms in GSTμ and cytochrome P4501A2 are associated with risk of large bowel cancer in population studies.

A more popular and longer established theory for the protective role of these foods is the fibre story originated by BURKITT (1971) [86]. BURKITT's original conten-

tion was that large bowel cancer risk was associated with low stool weights found in Western populations and this in turn was due to lack of dietary fibre. He also suggested a mechanism whereby fibre affected bacterial metabolism in the colon and transit time. BURKITT's theory was a truly seminal one, although it is quite clear now that not all aspects of it are correct. It has, however, proved to be the stimulus for a great deal of research and led to new insights into the bowel cancer story.

BURKITT's papers contained very little data, if any, on dietary fibre intakes in different populations although he did make some attempts to measure stool weights. Recently, however, it has been confirmed [13] that indeed there is an association between bowel habit and bowel cancer and that high stool weights are associated with protection against this condition (**Figure 26**).

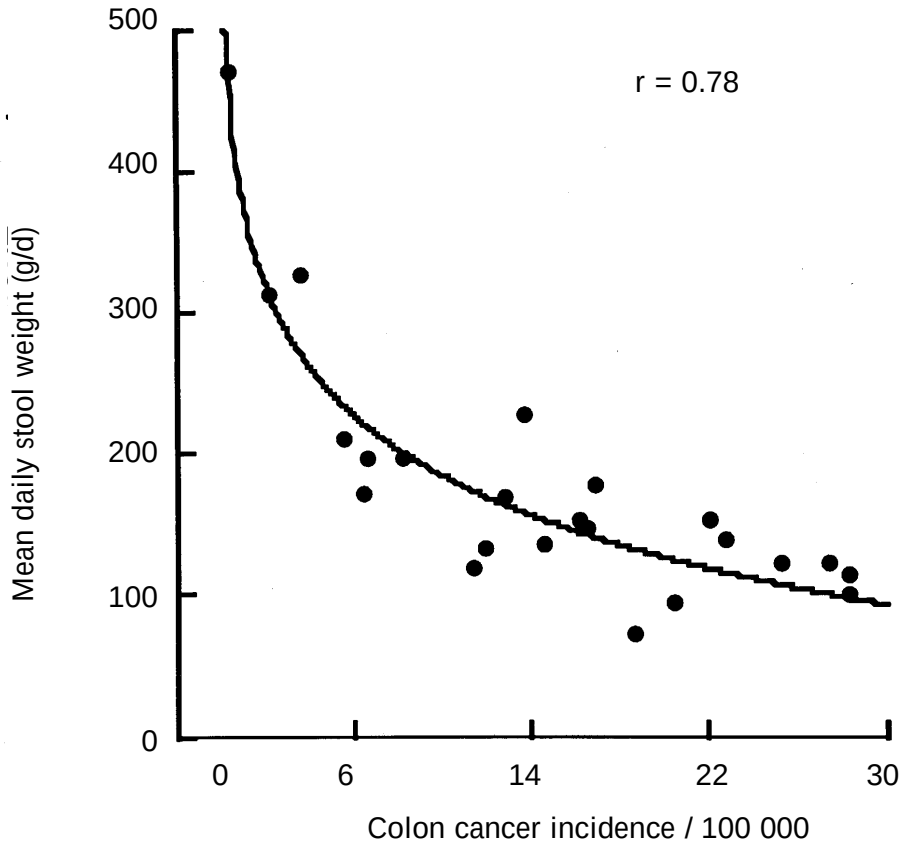

Figure 26 — Relationship between mean daily stool weight in 23 population groups worldwide and colon cancer incidence (from CUMMINGS *et al.* [13]).

The factors controlling bowel habit have been dealt with elsewhere in this monograph. Suffice it to say that stool weight is dependent, as far as diet is concerned, principally on the amount of fermentable carbohydrate that reaches

the colon. In addition to the non-starch polysaccharides of the plant cell wall (dietary fibre), other carbohydrates must be included in this theory including resistant starches and of course oligosaccharides. As yet there is very little epidemiological data to relate these potentially protective factors to large bowel cancer and such studies are needed.

How do fermentable carbohydrates exert their protective effect? There are a number of possible roles and these are listed in **table XXVII**. Perhaps the most important of them is the production of butyrate. The mechanisms whereby butyrate affect colonic epithelial cell function are described in detail in Chapter 4. Butyrate is a key substance in controlling differentiation and cell growth in the large intestine and may possibly effect DNA repair. A number of genes have now been identified whose transcription is regulated by butyrate, probably both at transcriptional and post-transcriptional levels. The genes specifically involved are gadd 153, which is a member of the C/EBP family of transcription factors, and BRF 1 and 2 [356, 623].

Fermentation may also affect bile acid metabolism and, in particular, resistant starch has been shown to reduce the levels of secondary bile acids in faecal water [107, 624].

Table XXVII — Colo-rectal cancer mechanisms.

Protective properties of fermented carbohydrate
– Butyrate – differentiating and gene regulatory effects
– Laxative effects – dilution of carcinogens
– Bile acids – reduced secondary bile acids
– Increased N uptake into bacteria
– Effects on carcinogen metabolism

8-5 Conclusion

There is ample evidence that colorectal cancer risks are determined by environmental factors acting at a genetic level. Diet is the best identified environmental factor at the moment, with fat and meat increasing risk and protective factors including any fermentable carbohydrate, together with antioxidant, nutrients and vitamins. Clear mechanisms have now been proposed to link the dietary components to the genetic elements in this condition, the whole theory providing a strong case for public health policy in this area.

References

1 HUNGATE R.E., PHILLIPS G.D., MCGREGOR A., HUNGATE D.O., BUECHNER H.K. Microbial fermentation in certain mammals. *Science* 1959;130:1192-1194.

2 CUMMINGS J.H., BANWELL J.G., SEGAL I., COLEMAN N., ENGLYST H.N., MACFARLANE G.T. The amount and composition of large bowel contents in man. *Gastroenterology* 1990;98:A408.

3 CUMMINGS J.H. Fermentation in the human large intestine: evidence and implications for health. *Lancet* 1983;1:1206-1209.

4 CUMMINGS J.H., MACFARLANE G.T. The control and consequences of bacterial fermentation in the human colon. *J. Appl. Bacteriol.* 1991;70:443-459.

5 MCNEIL N.I. The contribution of the large intestine to energy supplies in man. *Am. J. Clin. Nutr.* 1984;39:338-342.

6 PHILLIPS S.F., PEMBERTON J.H., SHORTER R.G., eds. *The large intestine: physiology, pathophysiology and disease.* New York: Raven Press, 1991:905.

7 GIBSON G.R., MACFARLANE G.T., eds. *Human colonic bacteria: role in nutrition, physiology and pathophysiology*. Boca Raton, Florida: CRC Press, 1995:292.

8 CUMMINGS J.H., ROMBEAU J.L., SAKATA T., eds. *Physiological and clinical aspects of short chain fatty acids*. Cambridge: Cambridge University Press, 1995:575.

9 ROWLAND I.R. *Role of the gut flora in toxicity and cancer*. London: Academic Press, 1988:517.

10 WRONG O.M., EDMONDS C.J., CHADWICK V.S., eds. *The large intestine: its role in mammalian nutrition and homeostasis.* Lancaster, UK: MTP Press, 1981:217.

11 KASPER H., GOEBELL H., eds. *Colon and Nutrition.* Lancaster, UK: MTP Press, 1982: 310 (Falk Symposium; 32).

12 RITCHIE J.K. The health of ileostomists. *Gut* 1971;12:536-540.

13 CUMMINGS J.H., BINGHAM S.A., HEATON K.W., EASTWOOD M.A. Fecal weight, colon cancer risk and dietary intake of non-starch polysaccharides (dietary fiber). *Gastroenterology* 1992;103:1783-1789.

14 BURKITT D.P., WALKER A.R.P., PAINTER N.S. Effect of dietary fibre on stools and transit times, and its role in the causation of disease. *Lancet* 1972;2:1408-1412.

15 METCALF A.M., PHILLIPS S.F., ZINSMEISTER A.R., MACCARTY R.L., BEART R.W., WOLFF B.G. Simplified assessment of segmental colonic transit. *Gastroenterology* 1987;92:40-47.

16 FINEGOLD S.M., SUTTER V.L. Fecal flora in different populations, with special reference to diet. *Am. J. Clin. Nutr.* 1978;31:S116-S122.

17 FINEGOLD S.M., SUTTER V.L., MATTISEN G.E. Normal indigenous intestinal flora. In: HENTGES D.J., ed. *Human Intestinal Microflora in Health and Disease*. New York: Academic Press, 1983: 3-31.

18 DRASAR B.S, HILL M.J. *Human intestinal flora*. London: Academic Press, 1974.

19 MOORE W.E.C., HOLDEMAN L.V. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Appl. Microbiol.* 1974;27:961-979.

20 MACFARLANE G.T., GIBSON G.R., DRASAR B.S., CUMMINGS J.H. Metabolic significance of the gut microflora. In: WHITEHEAD R., ed. *Gastrointestinal and Oesophageal Pathology*. 2nd ed. Edinburgh: Churchill Livingstone, 1995: 249-274.

21 SILVESTER K.R., ENGLYST H.N., CUMMINGS J.H. Ileal recovery of starch from whole diets containing resistant starch measured in vitro and fermentation of ileal effluent. *Am. J. Clin. Nutr.* 1995;62:403-411.

22 STEPHEN A.M., CUMMINGS J.H. Mechanism of action of dietary fibre in the human colon. *Nature* 1980;284:283-284.

23 PHILLIPS S.F., GILLER J. The contribution of the colon to electrolyte and water conservation in man. *J. Lab. Clin. Med.* 1973;81:733-746.

24 WRONG O., METCALFE-GIBSON A., MORRISON R.B.I., NG S.T., HOWARD A.V. In vivo dialysis of faeces as a method of stool analysis. I. Technique and results in normal subjects. *Clin. Sci.* 1965;28:357-.

25 CUMMINGS J.H. Nutritional management of diseases of the stomach and bowel. In: GARROW J.S., JAMES W.P.T, eds. *Davidson and Passmore's Human Nutrition & Dietetics.* 9th ed. Edinburgh: Churchill Livingstone, 1993: 480-506.

26 MACFARLANE G.T., GIBSON G.R., CUMMINGS J.H. Comparison of fermentation reactions in different regions of the human colon. *J. Appl. Bacteriol.* 1992;72:57-64.

27 CUMMINGS J.H., ENGLYST H.N. Fermentation in the large intestine and the available substrates. *Am. J. Clin. Nutr.* 1987;45:1243-1255.

28 CUMMINGS J.H., GIBSON G.R., MACFARLANE G.T. Quantitative estimates of fermentation in the hind gut of man. In: SKADHAUGE E., NORGAARD P., eds. *International Symposium on Comparative Aspects of the Physiology of Digestion in Ruminant and Hindgut Fermenters. Acta Vet. Scan.*, 1989: 76-82.

29 ENGLYST H., WIGGINS H.S., CUMMINGS J.H. Determination of the non-starch polysaccharides in plant foods by gas liquid chromatography of constituent sugars as alditol acetates. *Analyst* 1982;107:307-318.

30 ENGLYST H.N., KINGMAN S.M., CUMMINGS J.H. Classification and measurement of nutritionally important starch fractions. *Eur. J. Clin. Nutr.* 1992;46:S33-S50.

31 ENGLYST H.N., CUMMINGS J.H. Resistant starch, a 'new' food component: a classification of starch for nutritional purposes. In: MORTON I.D., ed. *Cereals in a European Context. First European Conference on Food Science & Technology*. Chichester, UK: Ellis Horwood, 1987: 221-233.

32 ENGLYST H.N., KINGMAN S.M. Dietary fiber and resistant starch: a nutritional classification of plant polysaccharides. In: KRITCHEVSKY D., BONFIELD C., ANDERSON J.W., eds. *Dietary Fiber: Chemistry, Physiology and Health Effects.* New York: Plenum, 1990: 48-65.

33 ROBERFROID M. Dietary fiber, inulin and oligofructose: a review comparing their physiological effects. *Crit. Rev. Food Sci. Nutr.* 1993;33 (2):103-148.

34 Department of Health. *Dietary sugars and human disease*. London: HMSO, 1989 (Reports on health and social subjects; 37).

35 HILL J.O., PRENTICE A.M. Sugar and body weight regulation. *Am. J. Clin. Nutr.* 1995;62:264S-274S.

36 CLYDESDALE F.M., ed. Nutritional and health aspects of sugars. *Am. J. Clin. Nutr.* 1995;62(1(S)):161S-296S.

37 SOUTHGATE D.A.T. Digestion and metabolism of sugars. *Am. J. Clin. Nutr.* 1995;62:203S-211S.

38 CALLOWAY D.H., MURPHY E.L. The use of expired air to measure intestinal gas formation. *Ann. New York Acad. Sci.* 1968;150:82-95.

39 TADESSE K., SMITH D,. EASTWOOD M.A. Breath hydrogen (H2) and methane (CH4) excretion patterns in normal man and in clinical practice. Q. *J. Exp. Physiol.* 1980;65:85-97.

40 OKU T. Dietary fiber and new sugars. In: BABA S., AKERELE O., KAWAGUCHI Y., eds. *Natural Resources and Human Health - Plants of Medicinal and Nutritional Value*. Amsterdam: Elsevier, 1992: 159-172.

41 YAZAWA K., IMAI K., TAMURA Z. Oligosaccharides and polysaccharides specifically utilisable by bifidobacteria. *Chem. Pharm. Bull.* 1978;26:3306-3311.

42 YAZAWA K., TAMURA Z. Search for sugar sources for selective increase of bifido-bacteria. *Bifidobact. Microflora* 1982;1:39-44.

43 ROBERFROID M., GIBSON G.R., DELZENNE N. The biochemistry of oligofructose, a non-digestible fiber: an approach to calculate its caloric value. *Nutr. Rev.* 1993;51:137-146.

44 VAN LOO J., COUSSEMENT P., DE LEENHEER L., HOEBREGS H., SMITS G. On the presence of inulin and oligofructose as natural ingredients in the Western diet. *Crit. Rev. Food Sci. Nutr.* 1995;35(6):525-552.

45 HIDAKA H., EIDA T. The production of oligosaccharides utilizing sugar transfer action. *Bio Industry* 1984;1:5-13.

46 MCCANCE R.A., LAWRENCE R.D. *The carbohydrate content of foods*. London: HMSO, 1929 (Special Report Series of the Medical Research Council; 135).

47 NILSSON U., OSTE R., JAGERSTAD M., BIRKHED D. Cereal fructans: in vitro and in vivo studies on availability in rats and humans. *J. Nutr.* 1988;118:1325-1330.

48 OKU T., TOKUNAGA T., HOSOYA N. Non-digestibility of a new sweetener "Neosugar" in the rat. *J. Nutr.* 1984;114:1574-1581.

49 BACH-KNUDSEN K.E., HESSOV I. Recovery of inulin from Jerusalem artichoke (*Helianthus tuberosus* L.) in the small intestine of man. *Br. J. Nutr.* 1995;74:101-103.

50 RUMESSEN J.J., BODE S., HAMBERG O., GUDMAND-HOYER E. Fructans of Jerusalem artichokes: intestinal transport, absorption, fermentation and influence on blood glucose, insulin and C-peptide responses in healthy subjects. *Am. J. Clin. Nutr.* 1990;52:675-681.

51 HEUPKE W., BLANCKENBURG K. Die Verdauung des Inulins. *Dtsch. Arch. Klin. Med.* 1934;176:182-187.

52 GIBSON G.R., BEATTY E.R., WANG X., CUMMINGS J.H. Selective stimulation of Bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* 1995;108:975-982.

53 HIDAKA H., EIDA T., TAKIZAWA T., TOKUNAGA T., TASHIRO Y. Effects of fructooligosaccharides on intestinal flora and human health. *Bifidobact. Microflora* 1986;5:37-50.

54 WANG X., GIBSON G.R. Effects of the in vitro fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *J. Appl. Bacteriol.* 1993;75:373-380.

55 MITSUOKA T., HIDAKA H., EIDA T. Effect of fructooligosaccharides on intestinal microflora. *Nahrung* 1987;31:5-6.

56 GIBSON G.R., WANG X. Regulatory effects of bifidobacteria on the growth of other colonic bacteria. *J. Appl. Bacteriol.* 1994;77:412-420.

57 KOK N., ROBERFROID M., DELZENNE N. Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. *Br. J. Nutr.* 1996;75:327-337.

58 FIORDALISO M., KOK N., DESAGER J.P. *et al.* Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats. *Lipids* 1995;30:163-167.

59 YAMASHITA K., KAWAI K., ITAKURA M. Effects of fructo-oligosaccharides on blood glucose and serum lipids in diabetic subjects. *Nutr. Res.* 1984;4:961-966.

60 BILIADERIS C.G. The structure and interactions of starch with food constituents. *Can. J. Physiol. Pharmacol.* 1991;69:60-78.

61 CUMMINGS J.H., ENGLYST H.N. Gastrointestinal effects of food carbohydrate. *Am. J. Clin. Nutr.* 1995;61:938S-945S.

62 ENGLYST H.N,. VEENSTRA J., HUDSON G.J. Measurement of rapidly available glucose (RAG) in plant foods: a potential in vitro predictor of the glycaemic response. *Br. J. Nutr.* 1996;75:327-337.

63 WAHLQVIST M.L., WILMSHURST E.G., MURTON C.R., RICHARDSON E.N. The effect of chain length on glucose absorption and the related metabolic response. *Am. J. Clin. Nutr.* 1978;31:1998-2001.

64 WONG S., O'DEA K. Importance of physical form rather than viscosity in determining the rate of starch hydrolysis in legumes. *Am. J. Clin. Nutr.* 1983;37:66-70.

65 CRAPO P.A., REAVEN G., OLEFSKY J. Plasma glucose and insulin responses to orally administered simple and complex carbohydrates. *Diabetes* 1976;25:741-747.

66 WURSCH P., DEL VEDOVO S., KOELLREUTTER B. Cell structure and starch nature as key determinants of the digestion rate of starch in legumes. *Am. J. Clin. Nutr.* 1986;43:25-29.

67 HERMANSEN K., RASMUSSEN O., ARNFRED J., WINTHER E., SCHMITZ O. Differential glycaemic effects of potato, rice and spaghetti in type 1 (insulin dependent) diabetic patients at constant insulinaemia. *Diabetalogia* 1986;29:358-361.

68 HEATON K.W., MARCUS S.N., EMMETT P.M., BILTON C.H. Particle size of wheat, maize and oat test meals: effects on plasma glucose and insulin responses and on the rate of starch digestion in vitro. *Am. J. Clin. Nutr.* 1988;47:675-682.

69 VAN DE WESTHUIZEN J., MBIZVO M., JONES J.J. Unrefined carbohydrate and glucose tolerance. *Lancet* 1972;ii:719.

70 HOLM J., BJORCK I., OSTROWSKA S., *et al.* Digestibility of amylose-lipid complexes in vitro and in vivo. *Starch/Starke* 1983;35:294-297.

71 SHAINKIN R., BIRK Y. Alpha-amylase inhibitors from wheat. Isolation and characterisation. *Biochim. Biophys. Acta* 1970;221:502-513.

72 DUNAIF G., SCHNEEMAN B.O. The effect of dietary fiber on human pancreatic activity in vitro. *Am. J. Clin. Nutr.* 1981;34:1034-1035.

73 READ N.W., WELCH I.M., AUSTEN C.J. *et al.* Swallowing food without chewing: a simple way to reduce postprandial glycaemia. *Br. J. Nutr.* 1986;55:43-47.

74 CHAPMAN R.W., SILLERY J.K. GRAHAM M.M., SAUNDERS D.R. Absorption of starch by healthy ileostomates: effect of transit time and of carbohydrate load. *Am. J. Clin. Nutr.* 1985;41:1244-1248.

75 ENGLYST H.N., CUMMINGS J.H. Digestion of the polysaccharides of some cereal foods in the human small intestine. *Am. J. Clin. Nutr.* 1985;42:778-787.

76 ENGLYST H.N., CUMMINGS J.H. Digestion of the polysaccharides of banana (Musa paradisiaca sapientum) in man. *Am. J. Clin. Nutr.* 1986;44:42-50.

77 ENGLYST H.N., CUMMINGS J.H. Digestion of the polysaccharides of potato in the small intestine of man. *Am. J. Clin. Nutr.* 1987;45:423-431.

78 ENGLYST H.N., KINGMAN S.M., HUDSON G.J., CUMMINGS J.H. Measurement of resistant starch in vitro and in vivo. *Br. J. Nutr.* 1996;75:749-755.

79 WOLEVER T.M.S., COHEN Z., THOMPSON L.U. *et al.* Ileal loss of available carbohydrate in man: comparison of a breath hydrogen method with direct measurement using a human ileostomy model. *Am. J. Gastroenterol.* 1986;81:115-122.

80 STEPHEN A.M., HADDAD A.C., PHILLIPS S.F. Passage of carbohydrate into the colon. Direct measurements in humans. *Gastroenterology* 1983;85:589-595.

81 LEVINE A.S., LEVITT M.D. Malabsorption of starch moiety of oats, corn and potatoes. *Gastroenterology* 1981;80:1209.

82 LEVITT M.D., HIRSCH P., FETZER C.A., SHEAHAN M., LEVINE A.S. H2 excretion after ingestion of complex carbohydrates. *Gastroenterology* 1987;92:383-389.

83 CUMMINGS J.H., ENGLYST H.N. Measurement of starch fermentation in the human large intestine. *Can. J. Physiol. Pharmacol.* 1991;69:121-129.

84 TROWELL H. Dietary fibre and coronary heart disease. *Eur. J. Clin. Biol. Res.* 1972;17:345-349.

85 HIGGINSON J., OETTLÉ A.G. Cancer incidence in the Bantu and "Cape Colored" races of South Africa: report of a cancer survey in the Transvaal (1953-55). *J. Natl. Cancer Inst.* 1960;24:589-671.

86 BURKITT D.P. Epidemiology of cancer of the colon and rectum. *Cancer* 1971;28:3-13.

87 TROWELL H.C. *Non-Infective Diseases in Africa.* London: Edward Arnold, 1960.

88 CLEAVE T.L., CAMPBELL A.D. *Diabetes, Coronary Thrombosis and the Saccharine Disease.* Bristol: John Wright, 1966.

89 PAINTER N.S. Diverticular disease of the colon: a disease of this century. *Lancet* 1969;2:586-588.

90 BURKITT D.P. Hemorrhoids, varicose veins and deep venous thrombosis: epidemiologic features and suggested causative factors. *Can. J. Surg.* 1975;18:483-488.

91 CUMMINGS J.H. Progress report: dietary fibre. *Gut* 1973;14:69-81.

92 REILLY R.W., KIRSNER J.B., eds. *Fiber Deficiency and Colonic Disorders.* New York: Plenum, 1975.

93 SPILLER G.A., AMEN R.J., eds. *Fiber in Human Nutrition*. New York: Plenum, 1976.

94 WILLIAMS R.D., OLMSTED W.H. The manner in which food controls the bulk of faeces. *Ann. Intern. Med.* 1936;10:717.

95 WILLIAMS R.D., OLMSTED W.H. The effect of cellulose, hemicellulose and lignin on the weight of the stool: a contribution to the study of laxation in man. *J. Nutr.* 1936;11:433.

96 STORY J.A., KRITCHEVSKY D. Dietary fiber and lipid metabolism. In: SPILLER G.A., AMEN R.J., eds. *Fiber in Human Nutrition*. New York: Plenum, 1976: 171-184.

97 SPILLER G.A., ed. *CRC Handbook of Dietary Fiber in Human Nutrition*. 2d ed. Boca Raton, Florida: CRC Press, 1993:

98 KRITCHEVSKY D., BONFIELD C., eds. *Dietary fiber in health and disease.* St. Paul, Minnesota: Eagan Press, 1995:1-486.

99 SOUTHGATE D.A.T. Determination of carbohydrates in foods. II. Unavailable carbohydrates. *J. Sci. Food Agric.* 1969;20:331-335.

100 VAN SOEST P.J., MCQUEEN R.W. The chemistry and estimation of fibre. *Proc. Nutr. Soc.* 1973;32:123-130.

101 TROWELL H., BURKITT D., HEATON K.W., eds. *Dietary Fibre, Fibre-Depleted Foods and Disease*. London: Academic Press, 1985.

102 TROWELL H. Dietary fibre: a paradigm. In: TROWELL H., BURKITT D., HEATON K.W., eds. *Dietary Fibre, Fibre-Depleted Foods and Diseases*. London: Academic Press, 1985: 1-20.

103 MCCANCE R.A., WIDDOWSON E.M. *The chemical composition of foods.* London: HMSO, 1940 (Special Report Series of the Medical Research Council; 235).

104 SELVENDRAN R.R., ROBERTSON J.A. The chemistry of dietary fibre - an holistic view of the cell wall matrix. In: SOUTHGATE D.A.T., WALDRON K., JOHNSON I.T., FENWICK G.R., eds. *Dietary Fibre: Chemical and Biological Aspects*. Cambridge, UK: Royal Society of Chemistry, 1990: 27-43.

105 CUMMINGS J.H. Dietary fibre. *Br. Med. Bull.* 1981;37:65-70.

106 CUMMINGS J.H., EDWARDS C., GEE J.M., NAGENGAST F.M., MATHERS J.C. Physiological effects of resistant starch in the large bowel. In: ASP N.-G., VAN AMELSVOORT

J.M.M., HAUTVAST J.G.A.J., eds. *Proceedings of the concluding plenary meeting of EURESTA*. Den Haag: European Commission - DG XII, 1995 (European Flair-Concerted Action Publication No.11 (Cost 911)).

107 VAN MUNSTER I.P., TANGERMAN A., NAGENGAST F.M. The effect of resistant starch on colonic fermentation, bile acid metabolism and mucosal proliferation. *Dig. Dis. Sci*. 1994;39:834-842.

108 PHILLIPS J., MUIR J.G., BIRKETT A., *et al.* Effect of resistant starch on fecal bulk and fermentation-dependent events in humans. *Am. J. Clin. Nutr.* 1995;62:121-130.

109 CUMMINGS J.H., BEATTY E.R., KINGMAN S.M., BINGHAM S.A., ENGLYST H.N. Digestion and physiological properties of resistant starch in the human large bowel. *Br. J. Nutr.* 1996;75:733-747.

110 PROSKY L., ASP N.-G., FURDA I., DEVRIES J.W., SCHWEIZER T.F., HARLAND B.F. Vitamins and other nutrients. Determination of total dietary fiber in foods, food products and total diets: interlaboratory study. *J. Assoc. Off. Anal. Chem.* 1984; 67:1044-1052.

111 PROSKY L., ASP N.-G., FURDA I., DEVRIES J.W., SCHWEIZER T.F., HARLAND B.F. Vitamins and other nutrients. Determination of total dietary fiber in foods and food products: collaborative study. *J. Assoc. Off. Anal. Chem.* 1985;68:677-679.

112 PROSKY L., ASP N.-G., SCHWEIZER T.F., DEVRIES J.W., FURDA I. Determination of insoluble, soluble and total dietary fiber in foods and food products: interlaboratory study. *J. Assoc. Off. Anal. Chem.* 1988;71:1017-1023.

113 LEE S.C., PROSKY L., DEVRIES J.W. Determination of total, soluble and insoluble dietary fiber in foods – enzymatic-gravimetric method, MES-TRIS buffer: collaboration study. *J. AOAC Int.* 1992;75:395-416.

114 PROSKY L., ASP N.-G., SCHWEIZER T.F., DEVRIES J.W., FURDA I. Determination of insoluble and soluble dietary fiber in foods and food products: collaborative study. *J. AOAC Int.* 1992;75:360-367.

115 JAMES W.P.T., THEANDER O., eds. *The Analysis of Dietary Fibre in Food.* New York: Marcel Dekker, 1981.

116 MONGEAU R., BRASSARD R. Collaborative study of a rapid gravimetric method. *Cereal Foods World* 1990;35:319-324.

117 MONGEAU R., BRASSARD R. A rapid method for the determination of soluble and insoluble dietary fiber: comparison with AOAC total dietary fiber procedure and Englyst's method. *J. Food Sci.* 1986;51:1333-1336.

118 SCHWEIZER T.F., WALTER E., VENETZ P. Collaborative study for the enzymatic, gravimetric determination of total dietary fibre in foods. *Mitt. Geb. Lebensmittel-unters. Hyg.* 1988;79:57-68.

119 CUMMINGS J.H., ENGLYST H.N., WOOD R. Determination of dietary fibre in cereals and cereal products - collaborative trials. Part I: Initial trial. *J. Assoc. Public Anal.* 1985;23:1-35.

120 WOOD R., ENGLYST H.N., SOUTHGATE D.A.T., CUMMINGS J.H. Determination of dietary fibre. Collaborative trials. Part IV. A comparison of the Englyst and Prosky

(AOAC) procedures for the determination of soluble, insoluble and total dietary fibre. *J. Assoc. Public Anal.* 1993;29:57-141.

121 ENGLYST H.N., QUIGLEY M.E., ENGLYST K.N., BRAVO L., HUDSON G.J. Dietary Fibre; Measurement by the Englyst NSP proceedure, Measurement by the AOAC proceedure, Explanation of the differences. *J. Assoc. Public Anal.* 1996; in press.

122 JERACI J.L., VAN SOEST P.J. Improved methods for analysis and biological characterization of fiber. In: FURDA I., BRINE C.J., eds. *New Developments in Dietary Fiber.* New York: Plenum, 1990: 245-263.

123 MEUSER F., SUCKOW P., KULIKOWSKI W. Verfahren zur Bestimmung von unloslichen und loslichen Ballaststoffen in Lebensmitteln. *Z. Lebensmittelunters. Forsch.* 1985;181:101-106.

124 THEANDER O., AMAN P. Studies on dietary fibre. A method for the analysis and chemical characterisation of total dietary fibre. *J. Sci. Food Agric.* 1982; 33:340-344.

125 THEANDER O., WESTERLUND E. Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. *J. Agric. food Chem.* 1986;34:330-336.

126 THEANDER O., AMAN P. Studies on dietary fibres. I. Analysis and chemical characterisation of water-soluble and water-insoluble dietary fibres. *Swed. J. Agric. Res.* 1979;9:97-106.

127 ENGLYST H.N., HUDSON G.J. Colorimetric method for routine measurement of dietary fibre as non-starch polysaccharides. A comparison with gas-liquid chromatography. *Food Chem.* 1987;24:63-76.

128 ENGLYST H.N., CUMMINGS J.H. Improved method for measurement of dietary fiber as non-starch polysaccharides in plant foods. *J. Assoc. Off. Anal. Chem.* 1988;71:808-814.

129 ENGLYST H.N., CUMMINGS J.H., Wood R. Determination of dietary fibre in cereals and cereal products - collaborative trials. Part II: Study of a modified Englyst procedure. *J. Assoc. Public Anal.* 1987;25:59-71.

130 ENGLYST N.H., CUMMINGS J.H., WOOD R. Determination of dietary fibre in cereals and cereal products - collaborative trials. Part III: Study of further simplified procedures. *J. Assoc. Public Anal.* 1987;25:73-110.

131 SOUTHGATE D.A.T., HUDSON G.J., ENGLYST H. The analysis of dietary fibre - the choices for the analyst. *J. Sci. Food. Agric.* 1978;29:979-988.

132 ENGLYST H., WIGGINS H. Measurement of the carbohydrate component of dietary fibre (non-starch polysaccharides). *Gut* 1979;20:A935.

133 QUIGLEY M.E., ENGLYST H.N. Determination of neutral sugars and hexosamines by high-performance liquid chromatography with pulsed amperometric detection. *Analyst* 1992;117:1715-1718.

134 Quigley M.E., Englyst H.N. Determination of the uronic acid constituents of non-starch polysaccharides by high-performance liquid chromatography with pulsed amperometric detection. *Analyst* 1994;119:1511-1518.

135 ENGLYST H.N., QUIGLEY M.E., HUDSON G.J. Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst* 1994;119:1497-1509.

136 ASP N.-G., SCHWEIZER T.F., SOUTHGATE D.A.T., THEANDER O. Dietary fibre analysis. In: SCHWEIZER T.F., EDWARDS C.A., eds. *Dietary Fibre - A Component of Food. Nutritional Function in Health and Disease*. Springer-Verlag, 1992: 57-101.

137 CUMMINGS J.H., FROLICH W., eds. *Dietary fibre intakes in Europe: a survey conducted by members of the Management Committee of COST 92. Metabolic and Physiological Aspects of Dietary Fibre in Food*. Brussels: ECSP-EEC-EAEC, 1993:1-89.

138 BINGHAM S. The dietary assessment of individuals: methods, accuracy, new techniques and recommendations. *Nut. Abst. Rev.* 1987;57:706-742.

139 ADIOTOMRE J.A., EASTWOOD M.A., EDWARDS C.A., BRYDON W.G. Dietary fiber: in vitro methods that anticipate nutrition and metabolic activity in humans. *Am. J. Clin. Nutr.* 1990;52:128-134.

140 SOUTHGATE D.A.T., WALDRON K., JOHNSON I.T., FENWICK G.R., eds. *Dietary Fibre: Chemical and Biological Aspects.* Cambridge: Royal Society of Chemistry, 1990.

141 EASTWOOD M.A. Vegetable fibre: its physical properties. *Proc. Nutr. Soc.* 1973;32:137-143.

142 MCCONNELL A.A., EASTWOOD M.A., MITCHELL W.D. Physical characteristics of vegetable foodstuffs that could influence bowel function. *J. Sci. Food Agric.* 1974;25:1457-1464.

143 ROBERTSON J.A., EASTWOOD M.A. An examination of factors which may affect the water holding capacity of dietary fibre. *Br. J. Nutr.* 1981;45:83-88.

144 BRODRIBB A.J.M., GROVES C. Effect of bran particle size on stool weight. *Gut* 1978;19:60-63.

145 STEPHEN A.M., CUMMINGS J.H. Water-holding by dietary fibre in vitro and its relationship to faecal output in man. *Gut* 1979;20:722-729.

146 GIBSON J.A., SLADEN G.E., DAWSON A.M. Protein absorption and ammonia production: the effects of dietary protein and removal of the colon. *Br. J. Nutr.* 1976;35:61-65.

147 CHACKO A., CUMMINGS J.H. Nitrogen losses from the human small bowel: obligatory losses and the effect of physical form of food. *Gut* 1988;29:809-815.

148 SILVESTER K.R., CUMMINGS J.H. Does digestibility of meat protein help explain large bowel cancer risk? *Nutr. Cancer* 1995;24:279-288.

149 FLORIN T.H.J., NEALE G., CUMMINGS J.H. The effect of dietary nitrate on nitrate and nitrite excretion in man. *Br. J. Nutr.* 1990;64:387-397.

150 SANDBERG A.S., ANDERSSON G.H., HALLGREN B., HASSELBLAD K., ISAKSSON B. Experimental model for in vivo determination of nutrients in the small intestine. *Br. J. Nutr.* 1981;45:283-294.

151 SANDBERG A.S., AHDERINNE R., ANDERSSON H., HALLGREN B., HULTEN I. The effect of citrus pectin on the absorption of nutrients in the small intestine. *Hum. Nutr. Clin. Nutr.* 1983;37C:171-183.

152 SMITH A.C., PODOLSKY D.K., eds. *Colonic mucin glycoproteins in health and disease.* Philadelphia: WB Saunders Company, 1986:815-837. (Mendeloff A., ed. Clinics in gastroenterology; vol. 15).

153 HOSKINS L.C., BOULDING E.T. Mucin degradation in human colon ecosystems. *J. Clin. Invest.* 1981;67:163-172.

154 HOSKINS L.C., AGUSTINES M., MCKEE W.B., BOULDING E.T., KRIARIS M., NIEDERMEYER G. Mucin degradation in human colon ecosystems. Isolation and properties of fecal strains that degrade ABH blood group antigens and oligosaccharides from mucin glycoproteins. *J. Clin. Invest.* 1985;75:944-953.

155 MILLER T.L., WEAVER G.A., WOLIN M.J. Methanogens and anaerobes in a colon segment isolated from the normal fecal stream. *Appl. Environ. Microbiol.* 1984;48:449-450.

156 ROBERTON A.M., STANLEY R.A. In vitro utilization of mucin by Bacteroides fragilis. *Appl. Environ. Microbiol.* 1982;43:325-330.

157 SALYERS A.A., O'BRIEN M., KOTARSKI S.F. Utilization of chrondroitin sulfate by Bacteroides thetaiotaomicron growing in carbohydrate-limited continuous culture. *J. Bacteriol.* 1982;150:1008-1015.

158 GIBSON G.R., CUMMINGS J.H., MACFARLANE G.T. Competition for hydrogen between sulphate-reducing bacteria and methanogenic bacteria from the human large intestine. *J. Appl. Bacteriol.* 1988;65:241-247.

159 CUMMINGS J.H. Short chain fatty acids in the human colon. *Gut* 1981;22:763-779.

160 ROCHE A.F., SILVERMAN E., eds. *Short-Chain Fatty Acids: Metabolism and Clinical Importance.* Columbus, Ohio: Ross Laboratories, 1991.

161 BINDER H.J., CUMMINGS J.H., SOERGEL K.H., eds. *Short-Chain Fatty Acids.* Dordrecht, The Netherlands: Kluwer Academic, 1994 (Falk Symposium; 73).

162 BUGAUT M. Occurrence, absorption and metabolism of short-chain fatty acids in the digestive tract of mammals. *Comp. Biochem. Physiol.* 1987;86:439-472.

163 BUGAUT M., BENTEJAC M. Biological effects of short-chain fatty acids in nonruminant mammals. *Annu. Rev. Nutr.* 1993;13:217-241.

164 CUMMINGS J.H,. POMARE E.W., BRANCH W.J., NAYLOR C.P.E., MACFARLANE G.T. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987;28:1221-1227.

165 WEAVER G.A., KRAUSER J.A., MILLER T.L., WOLIN M.J. Short chain fatty acid distributions of enema samples from a sigmoidoscopy population: an association of high acetate and low butyrate ratios with adenomatous polyps and colon cancer. *Gut* 1988;29:1539-1543.

166 MITCHELL B.L., LAWSON M.J., DAVIES M. *et al.* Volatile fatty acids in the human intestine: studies in surgical patients. *Nutr. Res.* 1985;5:1089-1092.

167 ELSDEN S.R., HITCHCOCK M.W.S., MARSHALL R.A., PHILLIPSON A.T. Volatile acid in the digesta of ruminants and other animals. *J. Exp. Biol.* 1946;22:191-202.

168 BREVES G., STUCK K. Short-chain fatty acids in the hindgut. In: CUMMINGS J.H, ROMBEAU J.L., SAKATA T., eds. *Physiological and Clinical Aspects of Short-Chain Fatty Acids.* Cambridge, UK: Cambridge University Press, 1995: 73-.

169 ODELSON D.A., BREZNAK J.A. Volatile fatty acid production by the hindgut microbiota of xylophagous termites. *Appl. Environ. Microbiol.* 1983;45:1602-1613.

170 MCBURNEY M.I. Passage of starch into the colon of humans: quantitation and implications. *Can. J. Physiol. Pharmacol.* 1991;69:130-136.

171 TITGEMEYER E.C., BOURQUIN L.D., FAHEY G.C.J., GARLEB K.A. Fermentability of various fiber sources by human fecal bacteria in vitro. *Am. J. Clin. Nutr.* 1991;53:1418-1424.

172 ENGLYST H.N., HAY S., MACFARLANE G.T. Polysaccharide breakdown by mixed populations of human faecal bacteria. *FEMS Microbiol. Ecol.* 1987;95:163-171.

173 WEAVER G.A., KRAUSE J.A., MILLER T.L., WOLIN M.J. Constancy of glucose and starch fermentations by two different human faecal microbial communities. *Gut* 1989;30:19.

174 WEAVER G.A., KRAUSE J.A., MILLER T.L., WOLIN M.J. Cornstarch fermentation by the colonic microbial community yields more butyrate than does cabbage fiber fermentation; cornstarch fermentation rates correlate negatively with methanogenesis. *Am. J. Clin. Nutr.* 1992;55:70-77.

175 MCBURNEY M.I., THOMPSON L.U. Effect of human faecal inoculum on in vitro fermentation variables. *B. J. Nutr.* 1987;58:233.

176 MCBURNEY M.I., THOMPSON L.U. In vitro fermentabilities of purified fiber supplements. *J. Food Sci.* 1989;54:347-350.

177 GIBSON G.R., MACFARLANE S., CUMMINGS J.H. The fermentability of polysaccharides by mixed human faecal bacteria in relation to their suitability as bulk forming laxatives. *Lett. Appl. Microbiol.* 1990;11:251-254.

178 MACFARLANE G.T., ENGLYST H.N. Starch utilization by the human large intestinal microflora. *J. Appl. Bacteriol.*1986;60:195-201.

179 ETTERLIN C., MCKEOWN A., BINGHAM S.A., ELIA M., MACFARLANE G.T., CUMMINGS J.H. D-Lactate and acetate as markers of fermentation in man. *Gastroenterology* 1992;102:A551.

180 TORRES-PINEDO R., LAVASTIDA M., RIVERA C.L., RODRIGUEZ H., ORTIZ A. Studies on infant diarrhea. 1. A comparison of the effects of milk feeding and intravenous therapy upon the composition and volume of the stool and urine. *J. Clin. Invest.* 1966;45:469-480.

181 WEIJERS H.A., VAN DE KAMER J.H., DICKE W.K., IJSSELING J. Diarrhoea caused by deficiency of sugar splitting enzymes. *Acta Paediatr. Scand.* 1961;50:55-71.

182 WEIJERS H.A., VAN DE KAMER J.H. Aetiology and diagnosis of fermentative diarrhoeas. *Acta Paediatr. Scand.* 1963;52:329-337.

183 Hoverstad T., Carlstedt-Duke B., Lingaas E. *et al.* Influence of ampicillin, clindamycin, and metronidazole on faecal excretion of short chain fatty acids in healthy subjects. *Scan. J. Gastroenterol.* 1986;21:621-626.

184 Hoverstad T., Carlstedt-Duke B., Lingaas E. *et al.* Influence of oral intake of seven different antibiotics on faecal short chain fatty acid excretion in healthy subjects. *Scan. J. Gastroenterol.* 1986;21:997-1003.

185 Heimdahl A., Nord C.E. Effect of erythromycin and clindamycin on the indigenous human anaerobic flora and new colonization of the gastrointestinal tract. *Eur. J. Clin. Microbiol.* 1982;1:38-48.

186 Edwards Ca., Duerden BI., Read N.W. The effect of clindamycin on the ability of a continuous culture of colonic bacteria to ferment carbohydrate. *Gut* 1986;27:411-417.

187 Clausen M.R., Bonnen H., Tvede M., Mortensen P.B. Colonic fermentation to short chain fatty acids is decreased in antibiotic-associated diarrhea. *Gastroenterology* 1991;101:1497-1504.

188 Rubinstein R., Howard A.V., Wrong O.M. In vivo dialysis of faeces as a method of stool analysis. IV. The organic anion component. *Clin. Sci.* 1969;37:549-564.

189 Wilson D.R., Ing T.S., Metcalfe-Gibson A., Wrong O.M. In vivo dialysis of faeces as a method of stool analysis. III. The effect of intestinal antibiotics. *Clin. Sci.* 1968;34:211-221.

190 Saunders D.R., Wiggins H.S. Conservation of mannitol, lactulose and raffinose by the human colon. *Am. J. Physiol.* 1981;241:G397-G402.

191 Cummings J.H., James W.P.T., Wiggins H.S. Role of the colon in ileal resection diarrhoea. *Lancet* 1973;i:344-347.

192 Grove E.W., Olmsted W.H., Koenig K. The effect of diet and catharsis on the lower volatile fatty acids in the stools of normal men. *J. Biol. Chem.* 1929/30;85: 127-136.

193 Cummings J.H., Hill M.J., Jenkins D..JA, Pearson J.R, Wiggins H.S. Changes in faecal composition and colonic function due to cereal fibre. *Am. J. Clin. Nutr.* 1976;29:1468-1473.

194 Olmsted W.H., Curtis G., Timm O.K. Stool volatile fatty acids. IV. The influence of feeding ban pentosan and fibre to man. *J. Biol. Chem.* 1935;108:645-652.

195 Spiller G.A., Chernoff M.C., Hill R.A., Gates J.E., Nassar J.J., Shipley E.A. Effect of purified cellulose, pectin, and a low-residue diet on fecal volatile fatty acids, transit time and fecal weight in humans. *Am. J. Clin. Nutr.* 1980;33:754-759.

196 Fleming S.E., Rodriguez M.A. Influence of dietary fibre in faecal excretion of volatile fatty acids by human adults. *J. Nutr.* 1983;113:1613-1626.

197 Fleming S.E. Influence of dietary fiber on the production, absorption, or excretion of short chain fatty acids in humans. In: Spiller G.A., ed. *CRC Handbook of Dietary Fiber in Human Nutrition.* 2d ed. Boca Raton, Florida: CRC Press, 1993: 387-412.

198 SCHEPPACH W., FABIAN C., SACHS M., KASPER H. The effect of starch malabsorption on fecal short chain fatty acid excretion in man. *Scan. J. Gastroenterol.* 1988;23:755-759.

199 CASSIDY A., BINGHAM S.A., CUMMINGS J.H. Starch intake and colorectal cancer risk: an international comparison. *Br. J. Cancer* 1994;69:937-942.

200 MACFARLANE G.T., GIBSON G.T. Microbiological aspects of short chain fatty acid production in the large bowel. In: CUMMINGS J.H., ROMBEAU J.L., SAKATA T., eds. *Physiological and Clinical Aspects of Short-Chain Fatty Acids.* Cambridge: Cambridge University Press, 1995: 87-106.

201 ISAACSON H.R., HINDS F.C., BRYANT M.P., OWENS F.N. Efficiency of energy utilization by mixed rumen bacteria in continuous culture. *J. Dairy Sci.* 1975; 58:1645-1659.

202 OWENS F.N., ISAACSON H.R. Ruminal microbial yields: factors influencing synthesis and bypass. *Fed. Proc.* 1977;36:198-202.

203 STEPHEN A.M., WIGGINS H.S., CUMMINGS J.H. Effect of changing transit time on colonic microbial metabolism in man. *Gut* 1987;28:601-609.

204 ALLISON C., MCFARLAN C., MACFARLANE G.T. Studies on mixed populations of human intestinal bacteria grown in single and multistage continuous culture systems. *Appl. Environ. Microbiol.* 1989;55:672-678.

205 MACFARLANE G.T., CUMMINGS J.H., MACFARLANE S., GIBSON G.R. Influence of retention time on degradation of pancreatic enzymes by human colonic bacteria grown in a 3-stage continuous culture system. *J. Appl. Bacteriol.* 1989;67:521-527.

206 CUMMINGS J.H. Short-chain fatty acids. In: GIBSON G.R., MACFARLANE G.T., eds. *Human Colonic Bacteria: Role in Nutrition, Physiology and Pathology.* Boca Raton, Florida: CRC Press, 1995: 101-130.

207 DAVIGNON J., SIMMONDS W.J., AHRENS E.H. Usefulness of chromic oxide as an internal standard for balance studies in formula-fed patients and for assessment of colonic function. *J. Clin. Invest.* 1968;47:127-138.

208 CUMMINGS J.H. Diet and transit through the gut. *J. Plant Foods* 1978;3:83-95.

209 CUMMINGS J.H., HILL M.J., BONE E.S., BRANCH W.J,. JENKINS D.J.A. The effect of meat protein and dietary fiber on colonic function and metabolism. Part II. Bacterial metabolites in faeces and urine. *Am. J. Clin. Nutr.* 1979;32:2094-2101.

210 MACY I.G. *Nutrition and Chemical Growth in Childhood.* Baltimore: C.C.Thomas, 1942 (vol. 1, Evaluation).

211 BERGMAN E.N. Production and utilization of metabolites by the alimentary tract as measured in portal and hepatic blood. In: MCDONALD I.W., WARNER A.C.I., eds. *Digestion and metabolism in the ruminant.* Armidale, New South Wales (Australia): University of New England, 1975: 292-305.

212 BERGMAN E.N. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol. Rev.* 1990;70:567-590.

213 DANKERT J., ZIJLSTRA J.B., WOLTHERS B.G. Volatile fatty acids in human peripheral and portal blood: quantitative determination by vacuum distillation and gas chromatography. *Clin. Chim. Acta* 1981;110:301-307.

214 PETERS S.G., POMARE E.W., FISHER C.A. Portal and peripheral blood short chain fatty acid concentrations after caecal lactulose instillation at surgery. *Gut* 1992;33:1249-1252.

215 SKUTCHES C.L., HOLROYDE C.P., MYERS R.N., PAUL P., REICHARD G.A. Plasma acetate turnover and oxidation. *J. Clin. Invest.* 1979;64:708-713.

216 SCHEPPACH W., POMARE E.W., ELIA M., CUMMINGS J.H. The contribution of the large intestine to blood acetate in man. *Clin. Sci.* 1991;80:177-182.

217 SCHENK W.G., MCDONALD J.C., MCDONALD K., DRAPONAS T. Direct measurement of hepatic flow in surgical patients. *Ann. Surg.* 1962;156:463-469.

218 NORRYD C., DENCKER H., LUNDERQUIST A., OLIN T., TYLEN U. Superior mesenteric artery blood flow during digestion in man. *Acta Chir. Scand.* 1975;141:197-202.

219 QAMAR M.I., READ A.E. Effects of ingestion of carbohydrate, fat, protein and water on the mesenteric blood flow in man. *Scand. J. Gastroenterol.* 1988;23:26-30.

220 POMARE E.W., BRANCH W.J., CUMMINGS J.H. Carbohydrate fermentation in the human colon and its relation to acetate concentration in venous blood. *J. Clin. Invest.* 1985;75:1448-1454.

221 BRIDGES S.R., ANDERSON J.W., DEAKINS D.D., DILLON D.W., WOOD C.L. Oat bran increases serum acetate of hypercholesterolemic men. *Am. J. Clin. Nutr.* 1992;56:455-459.

222 NEILSEN L.G., OWEN-ASH K., THOR E. Gas chromatographic method for plasma acetate analysis in acetate intolerance studies. *Clin. Chem.* 1978;24:348-350.

223 AKANJI A.O., HUMPHREYS S., THURSFIELD V., HOCKADAY, T.D.R. The relationship of plasma acetate with glucose and other blood intermediary metabolites in non-diabetic and diabetic subjects. *Clin. Chim. Acta* 1989;185:25-34.

224 LIVESEY G., ELIA M. Short chain fatty acids as an energy source in the colon: metabolism and clinical implications. In: CUMMINGS J.H., ROMBEAU J.L., SAKATA T., eds. *Physiological and Clinical Aspects of Short-Chain Fatty Acids*. Cambridge, UK: Cambridge University Press, 1995: 427-482.

225 WOLIN M.J., MILLER T.L. Carbohydrate fermentation. In: HENTGES D.J., ed. *Human Intestinal Microflora in Health and Disease*. New York: Academic Press, 1983: 147-166.

226 WOLIN M.J. A theoretical rumen fermentation balance. *J. Dairy Sci.* 1960;43:1452-1459.

227 WOLIN M.J. Fermentation in the rumen and human large intestine. *Science* 1981;213:1463-1468.

228 MILLER T.L., WOLIN M.J. Fermentations by saccharolytic intestinal bacteria. *Am. J. Clin. Nutr.* 1979;32:164-172.

229 MATHERS J.C., ANNISON E.F. Stoichiometry of polysaccharide fermentation in the large intestine. In: *Chemistry and Nutrition of Dietary Fibre and Beyond: Australian Perspectives*. Australia: Australian Nutrition Society, 1993: 123-135 (Occasional Publication No. 1).

230 GIBSON G.R., MACFARLANE G.T., CUMMINGS J.H. Sulphate reducing bacteria and hydrogen metabolism in the human large intestine. *Gut* 1993;34:437-439.

231 WALTER J.H., LEONARD J.V., THOMPSON G.N., BARTLETT K., HALLIDAY D. Contribution of aminoacid catabolism to propionate production in methylmalonic acidaemia. *Lancet* 1989;i:1298-1299.

232 BREVES G., SCHULZE E., SALLMANN H.P., GADEKEN D. The application of 13C labelled short chain fatty acids to measure acetate and propionate production rates in the large intestines: studies in a pig model. *Z. Gastroenterol.* 1993;31:179-182.

233 FREEMAN K., FOY T., FESTE A.S., REEDS P.J., LIFSCHITZ C.L. Colonic acetate in the circulating acetate pool of the infant pig. *Paediatr. Res.* 1993;34:318-322.

234 KIEN C.L., KEPNER J., GROTJOHN K., AULT K., MCLEAD R.E. Stable isotope model for estimating colonic acetate production in premature infants. *Gastroenterology* 1992;102:1458-1466.

235 MACFARLANE G.T., GIBSON G.R., BEATTY E.R., CUMMINGS J.H. Estimation of short-chain fatty acid production from protein by human intestinal bacteria based on branched chain fatty acid measurements. *FEMS Microbiol. Ecol.* 1992; 101:81-88.

236 MCNEIL N.I., CUMMINGS J.H., JAMES W.P.T. Short chain fatty acid absorption by the human large intestine. *Gut* 1978;19:819-822.

237 RUPPIN H., BAR-MEIR S., SOERGEL K.H., WOOD C.M., SCHMITT M.G. Absorption of short-chain fatty acids by the colon. *Gastroenterology* 1980;78:1500-1507.

238 DAWSON A.M., HOLDSWORTH C.D., WEBB J. Absorption of short chain fatty acids in man. *Proc. Soc. Exp. Biol. Med.* 1964;117:97-100.

239 MCNEIL N.I., CUMMINGS J.H., JAMES W.P.T. Rectal absorption of short chain fatty acids in the absence of chloride. *Gut* 1979;20:400-403.

240 GABEL G., MARTENS H. Transport of Na+ and Cl- across the forestomach epithelium: mechanisms and interactions with short chain fatty acids. In: TSUDA T., SASAKI Y., KAWASHIMA R., eds. *Physiological Aspects of Digestion and Metabolism in Ruminants. Proceedings of the Seventh International Symposium on Ruminant Physiology*. New York: Academic Press, 1991: 129.

241 MARTENS H., LEONHARD S., GABEL G. Minerals and digestion: exchanges in the digestive tract. In: JOUANY J.P., ed. *Rumen Microbial Metabolism and Ruminant Digestion*. Versailles: INRA Editions, 1991: 199.

242 SOERGEL K.H., HARIG J.M., LOO F.D., RAMASWAMY K., WOOD C.M. Colonic fermentation and absorption of SCFA in man. *Acta Vet. Scand.* 1989;Suppl. 86:107-115.

243 VON ENGELHARDT W. Absorption of short-chain fatty acids from the large intestine. In: CUMMINGS J.H., ROMBEAU J.L., SAKATA T., eds. *Physiological and Clinical Aspects of Short-Chain Fatty Acids.* Cambridge, UK: Cambridge University Press, 1995: 149-170.

244 ARGENZIO R.A., SOUTHWORTH M., LOWE J.E., STEVENS C.E. Interrelationship of Na, HCO3 volatile fatty acid transport by equine large intestine. *Am. J. Physiol.* 1977;233:E468-E469.

245 ARGENZIO R.A., WHIPP S.C. Inter-relationship of sodium, chloride, bicarbonate and acetate transport by the colon of the pig. *J. Physiol.* 1979;295:365-381.

246 RUBSAMEN K., VON ENGELHARDT W. Absorption of Na, H ions and short chain fatty acids from the sheep colon. *Pflügers Arch.* 1981;391:141-146.

247 UMESAKI Y., YAJIMA T., YOKOKURA T., MUTAI M. Effect of organic acid absorption on bicarbonate transport in rat colon. *Pflügers Arch.* 1979;379:43-47.

248 MASCOLO N., RAJENDRAN V.M., BINDER H.J. Mechanism of short chain fatty acid uptake by apical membrane vesicles of rat distal colon. *Gastroenterology* 1991;101:331-338.

249 HARIG J.M., KNAUP S.M., SHOSHARA J., DUDEJA P.K., RAMASWAMY K., BRASITUS T.A. Transport of N-butyrate into human colonic luminal membrane vesicles. *Gastroenterology* 1990;98:A543.

250 STEVENS C.E., STETTLER B.K. Factors affecting the transport of volatile fatty acids across rumen epithelium. *Am. J. Physiol.* 1966;210:365-372.

251 LONNERHOLM G., SELKING O., WISTRAND P.J. Amount and distribution of carbonic anhydrases CA I and CA II in the gastrointestinal tract. *Gastroenterology* 1985;88:1151-1161.

252 CHARNEY A.N., WAGNER J.D., BIRNBAUM G.J., JOHNSTONE J.N. Functional role of carbonic anhydrase in intestinal electrolyte transport. *Am. J. Physiol.* 1986;251:G682-G687.

253 VON ENGELHARDT W., GROS G., BURMESTER M., HANSEN K., BECKER G., RECHKEMMER G. Functional role of bicarbonate in propionate transport across guinea pig large intestine. *J. Physiol.* 1994;477:365-371.

254 PARSONS D.S., PATERSON C.R. Fluid and solute transport across rat colonic mucosa. *Q. J. Exp. Physiol.* 1965;50:220-231.

255 ARGENZIO R.A., MILLER N., VON ENGELHARDT W. Effect of volatile fatty acids on water and ion absorption from the goat colon. *Am. J. Physiol.* 1975;229:997-1002.

256 CRUMP M.H., ARGENZIO R.A., WHIPP S.C. Effects of acetate on absorption of solute and water from the pig colon. *Am. J. Vet. Res.* 1980;41:1565-1568.

257 ROEDIGER W.E.W., MOORE A. The effect of short-chain fatty acids on sodium absorption in the isolated human colon perfused through the vascular bed. *Dig. Dis. Sci.* 1981;26:100-106.

258 UMESAKI Y., YAJIMA T., TOHYAMA K., MUTAI M. Characterization of acetate uptake by the colonic epithelial cells of the rat. *Pflügers Arch.* 1980;388:205-209.

259 ROEDIGER W.E.W., DEAKIN E.J., RADCLIFFE B.C., NANCE S. Anion control of sodium absorption in the colon. *J. Exp. Physiol.* 1986;71:195-204.

260 ROEDIGER W.E.W. Short chain fatty acids as metabolic regulators of ion absorption in the colon. *Acta Vet. Scand.* 1989;86:116-125.

261 ROEDIGER W.E.W. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut* 1980;21:793-798.

262 ROEDIGER W.E.W. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* 1982;83:424-429.

263 MARTY J., VERNAY M. Absorption and metabolism of the volatile fatty acids in the hind-gut of the rabbit. *Br. J. Nutr.* 1984;51:265-277.

264 VERNAY M. Effects of plasma aldosterone on butyrate absorption and metabolism in the rabbit proximal colon. *Comp. Biochem. Physiol.* 1987;86A:657-662.

265 ARDAWI M.S.M., NEWSHOLME E.A. Fuel utilisation in colonocytes of the rat. *Biochem. J.* 1985;231:713-719.

266 FLEMING S.E., FITCH M.D., DE VRIES S., LIU M.L., KIGHT C. Nutrient utilization by cells isolated from rat jejunum, cecum and colon. *J. Nutr.* 1991;121:869-878.

267 ROEDIGER W.E.W, KAPANIRIS O., MILLARD S. Lipogenesis from n-butyrate in colonocytes. Action of reducing agent and 5-aminosalicylic acid with relevance to ulcerative colitis. *Mol. Cell. Biochem.* 1992;118:113-118.

268 REMESY C., DEMIGNE C. Partition and absorption of volatile fatty acids in the alimentary canal of the rat. *Ann. Rech. Vét.* 1976;7:39-55.

269 HENNING S.J., HIRD F.J.R. Concentration and metabolism of volatile fatty acids in the fermentive organs of 2 species of kangaroo and the guinea-pig. *Br. J. Nutr.* 1970;24:146-155.

270 HENNING S.J., HIRD F.J.R. Ketogenesis from acetate and butyrate by the caecum and the colon of rabbits. *Biochem. J.* 1972;130:785-790.

271 ROEDIGER W.E.W. Cellular metabolism of short-chain fatty acids in colonic epithelial cells. In: ROCHE A.F., SILVERMAN E., eds. *Short Chain Fatty Acids: Metabolism and Clinical Importance*. Columbus, Ohio: Ross Laboratories, 1991: 67-70.

272 BROWN R.C., KELLEHER J., LOSOWSKY M.S. The effect of pectin on the structure and function of the rat small intestine. *Br. J. Nutr.* 1979;42:357-365.

273 JACOBS L.R. Effects of dietary fiber on mucosal growth and cell proliferation in the small intestine of the rat: a comparison of oat bran, pectin, and guar with total fiber deprivation. *Am. J. Clin. Nutr.* 1983;37:954-960.

274 SIGLEO S., JACKSON M.J., VAHOUNY G.V. Effects of dietary fiber constituents on intestinal morphology and nutrient transport. *Am. J. Physiol.* 1984;246:G34-G39.

275 JOHNSON I.T., GEE J.M., MAHONEY R.R. Effect of dietary supplements of guar gum and cellulose on intestinal cell proliferation, enzyme levels and sugar transport in the rat. *Br. J. Nutr.* 1984;52:477-487.

276 SAKATA T., YAJIMA T. Influence of short chain fatty acids on the epithelial cell division of digestive tract. *Q. J. Exp. Physiol.* 1984;69:639-648.

277 CASSIDY M.M., LIGHTFOOT F.G., GRAU L.E., STORY J.A., KRITCHEVSKY D., VAHOUNY G. Effect of chronic intake of dietary fibers on the ultrastructural topography of rat jejunum and colon: a scanning electron microscopy study. *Am. J. Clin. Nutr.* 1981;34:218-228.

278 JACOBS L.R., WHITE F.A. Modulation of mucosal cell proliferation in the intestine of rats fed a wheat bran diet. *Am. J. Clin. Nutr.* 1983;37:945-953.

279 JACOBS L.R., LUPTON J.R. Effect of dietary fibers on rat large bowel mucosal growth and cell proliferation. *Am. J. Physiol.* 1984;246:G378-G385.

280 JACOBS L.R., SCHNEEMAN B.O. Effects of dietary wheat bran on rat colonic structure and mucosal cell growth. *J. Nutr.* 1981;111:798-803.

281 SAKATA T, VON ENGELHARDT W. Stimulatory effect of short chain fatty acids on the epithelial cell proliferation in rat large intestine. *Comp. Biochem. Physiol.* 1983;74A:459-462.

282 GOODLAD R.A., LENTON W., GHATEI M.A., ADRIAN T.E., BLOOM S.R., WRIGHT N.A. Effects of an elemental diet, inert bulk and different types of dietary fibre on the response of the intestinal epithelium to refeeding in the rat and relationship to plasma gastrin, enteroglucagon and PYY concentrations. *Gut* 1987;28:171-180.

283 GOODLAD R.A., RATCLIFFE B., FORDHAM J.P., WRIGHT N.A. Does dietary fibre stimulate intestinal epithelial cell proliferation in germ free rats. *Gut* 1989;30:820-825.

284 SAKATA T. Short chain fatty acids as the luminal trophic factor. *Can. J. Anim. Sci.* 1984;64:189-190.

285 SAKATA T. Effects of indigestible dietary bulk and short chain fatty acids on the tissue weight and epithelial cell proliferation rate of the digestive tract in rats. *J. Nutr. Sci. Vitaminol.* 1986;32:355-362.

286 SAKATA T. Stimulatory effect of short chain fatty acids on epithelial cell proliferation in the rat intestine: a possible explanation for trophic effects of fermentable fibre, gut microbes and luminal trophic factors. *Br. J. Nutr.* 1987;58:95-103.

287 SAKATA T. Depression of intestinal epithelial cell production rate by hindgut bypass in rats. *Scand. J. Gastroenterol.* 1988;23:1200-1202.

288 JANNE P., CARPENTER Y., WILLEMS G. Colonic mucosal atrophy induced by a liquid elemental diet in rats. *Am. J. Dig. Dis.* 1977;22:808-812.

289 GOODLAD R.A., WRIGHT N.A. The effects of addition of cellulose or kaolin to an elemental diet on intestinal cell proliferation in the mouse. *Br. J. Nutr.* 1983;50:91-98.

290 KRIPKE S.A., FOX A.D., BERMAN J.M., SETTLE R.G., ROMBEAU J.L. Stimulation of intestinal mucosal growth with intracolonic infusion of short-chain fatty acids. *J. Parenter. Enter. Nutr.* 1989;13:109-116.

291 KORUDA M.J., ROLANDELLI R.H., BLISS D.Z., HASTINGS J., ROMBEAU J.L., SETTLE R.G. Parenteral nutrition supplemented with short chain fatty acids: effect on the small bowel mucosa in normal rats. *Am. J. Clin. Nutr.* 1990;51:685-689.

292 FRANKEL W.L., ZHANG W., SINGH A. *et al.* Mediation of the trophic effects of short-chain fatty acids on the rat jejunum and colon. *Gastroenterology* 1994;106:375-380.

293 JACOBS L.R., LUPTON J.R. Relationship between colonic luminal pH, cell proliferation and colon carcinogenesis in 1,2-dimethylhydrazine-treated rats fed high fiber diets. *Cancer Res.* 1986;46:1727-1734.

294 FREEMAN H.J. Effects of differing concentrations of sodium butyrate on 1,2-dimethylhydrazine-induced rat intestinal neoplasia. *Gastroenterology* 1986;91:596-602.

295 WATANABE K., REDDY B.S., WEISBURGER J.H., KRITCHEVSKY D. Effect of dietary alfalfa, pectin and wheat bran on azoxymethane- or methylnitrosourea-induced colon carcinogenesis in F344 rats. *J. Nat. Cancer Inst.* 1979;63:141-145.

296 SCHEPPACH W., BARTRAM P., RICHTER A. *et al.* Effect of short chain fatty acids on the human colonic mucosa in vitro. *J. Parenter. Enter. Nutr.* 1992;16:43-48.

297 SCHEPPACH W.M. Short chain fatty acids are a trophic factor for the human colonic mucosa in vitro. In: *Short Chain Fatty Acids: Metabolism and Clinical Importance.* Columbus, Ohio: Ross Laboratories, 1991: 90-93.

298 LIPKIN M., BLATTNER W.A., FRAUMENI J.F.J., LYNCH H.T., DESCHNER E.E., WINAWAR S. Tritiated thymidine labeling distribution in the identification of hereditary predisposition to colon cancer. *Cancer Res.* 1983;43:1899-1904.

299 PRASAD K.N., SINHA P.K. Effect of sodium butyrate on mammalian cells in culture: a review. *In Vitro* 1976;12:125-132.

300 PRASAD K.N. Butyric acid: a small fatty acid with diverse biological functions. *Life Sci.* 1980;27:1351-1358.

301 KRUH J. Effects of sodium butyrate, a new pharmacological agent, on cells in culture. *Mol. Cell. Biochem.* 1982;42:65-82.

302 KRUH J., DEFER N., TICHONICKY L. Action moleculaire et cellulaire du butyrate. *C.R. Soc. Biol.* 1992;186:12-25.

303 FREGEAU C.J., HELGASON C.D., BLEACKLEY R.C. Two cytotoxic cell proteinase genes are differentially sensitive to sodium butyrate. *Nucleic Acids Res.* 1992;20: 3113-3119.

304 SMITH P.J. n-Butyrate alters chromatin accessibility to DNA repair enzymes. *Carcinogenesis* 1986;7:423-429.

305 GLAUBER J.G., WANDERSEE N.J., LITTLE J.A., GINDER G.D. 5' flanking sequences mediate butyrate stimulation of embryonic globin gene expression in adult erythroid cells. *Mol. Cell. Biol.* 1991;11:4690-4697.

306 HAGUE A., MANNING A.M., HANLON K.A., HUSCHISCHA L.I., HART D., PARASKEVA C. Sodium butyrate induces apoptosis in human colonic tumour cell lines in a p53-independent pathway: implications for the possible role of dietary fibre in the prevention of large bowel cancer. *Int. J. Cancer* 1993;55:498-505.

307 HAGUE A., ELDER D.J.E., HICKS D.J., PARASKEVA C. Apoptosis in colorectal tomour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the biles alt deoxycholate. *Int. J. Cancer* 1995;60:400-406.

308 HAGOPIAN H.K., RIGGS M.G., SWARTZ L.A., INGRAM V.M. Effect of n-butyrate on DLA synthesis in chick fibroblasts and HeLa cells. *Cell* 1977;12:855-860.

309 D'ANNA J.A., TOBEY R.A., GURLEY L.R. Concentration-dependent effects of sodium butyrate in Chinese hamster cells: cell-cycle progression, inner histone

acetylation, histone H1 dephosphorylation and induction of an H1-like protein. *Biochemistry* 1980;19:2656-2671.

310 WINTERSBERGER E., MUDRAK I., WINTERSBERGER U. Butyrate inhibits mouse fibroblasts at a control point in the GI phase. *J. Cell. Biochem.* 1983;21:239-247.

311 TOSCANI A., SOPRANO D.R., SOPRANO K.J. Molecular analysis of sodium butyrate-induced growth arrest. *Oncogene Res.* 1988;3:223-238.

312 VAN WIJK R., TICHONICKY L., KRUH J. Effect of sodium butyrate on the hepatoma cell cycle: possible use for cell synchronization. *In Vitro* 1981;17:859-862.

313 FLATOW U., RABSON A.B., HAND P.H., WILLINGHAM M.C., RABSON A.S. Characterization and tumorigenicity of a butyrate-adapted T24 bladder cancer cell line. *Cancer Invest.* 1989;7:423-435.

314 GAMET L., DAVIAUD D., DENIS-POUXVIEL C., REMESY C., MURAT J.-C. Effects of short-chain fatty acids on growth and differentiation of the human colon-cancer cell line HT29. *Int. J. Cancer* 1992;52:286-289.

315 DEXTER D.L., LEV R., MCKENDALL G.R., MITCHELL P., CALABRES P. Sodium butyrate-induced alteration of growth properties and glycogen levels in cultured human colon carcinoma cells. *Histochem. J.* 1984;16:137-149.

316 KIM Y.S., TSAO D., SIDDIQUI B. *et al.* Effects of sodium butyrate and dimethylsulfoxide on biochemical properties of human colon cancer cells. *Cancer* 1980; 45:1185-1192.

317 WHITEHEAD R.H., YOUNG G.P., BHATHAL P.S. Effects of short chain fatty acids on a new human colon carcinoma cell line (LIM1215). *Gut* 1986;27:1457-1463.

318 TSAO D., MORITA A., BELLA A., LUU P., KIM Y.S. Differential effects of sodium butyrate, dimethyl sulfoxide and retinoic acid on membrane-associated antigen, enzymes and glycoproteins of human rectal adenocarcinoma cells. *Cancer Res.* 1982;42:1052-1058.

319 CHUNG Y.S., SONG I.S., ERICKSON R.H., SLEISINGER M.H., KIM Y.S. Effect of growth and sodium butyrate on brush border membrane-associated hydrolases in human colorectal cancer cell lines. *Cancer Res.* 1985;45:2976-2982.

320 GIBSON P.R., MOELLER I., KAGELARI O., FOLINO M., YOUNG G.P. Contrasting effects of butyrate on the expression of phenotypic markers of differentiation in neoplastic and non-neoplastic colonic epithelial cells in vitro. *J. Gastroenterol. Hepatol.* 1992;7:165-172.

321 BLOOM E.J., SIDDIQUI B., HICKS J.W., KIM Y.S. Effect of sodium butyrate, a differentiating agent, on cell surface glycoconjugates of a human pancreatic cell line. *Pancreas* 1989;4:59-64.

322 DYSON J.E.D., DANIEL J., SURREY C.R. The effect of sodium butyrate on the growth characteristics of human cervix tumour cells. *Br. J. Cancer* 1992;65:803-808.

323 NORDENBERG J., WASSERMAN L., BEERY E. *et al.* Growth inhibition of murine melanoma by butyric acid and dimethylsulfoxide. *Exp. Cell Res.* 1986;162:77-85.

324 NORDENBERG J., WASSERMAN L., PELED A., MALIK Z., STENZEL K.H., NOVOGRODSKY A. Biochemical and ultrastructural alterations accompany the anti-proliferative effect of butyrate on melanoma cells. *Br. J. Cancer* 1987;55:493-497.

325 RAMA B.N., PRASAD K.N. Modification of the hyperthermic response on neuroblastoma cells by cAMP and sodium butyrate. *Cancer* 1986;58:1448-1452.

326 REESE D.H., GRATZNER H.G., BLOCK N.L., POLITANO V.A. Control of growth, morphology and alkaline phosphatase activity by butyrate and related short-chain fatty acids in the retinoid-responsive 9-1C rat prostatic adenocarcinoma cell. *Cancer Res.* 1985;45:2308-2313.

327 HALGUNSET J., LAMVIK T., ESSPEVIK T. Butyrate effects on growth, morphology and fibronectin production in PC-3 prostatic carcinoma cells. *Prostate* 1988;12:65-77.

328 GUILBAUD N.F., GAS N., DUPONT M.A., VALETTE A. Effects of differentiation-inducing agents on maturation of human MCF-7 breast cancer cells. *J. Cell. Physiol.* 1990;145:162-172.

329 MORITA A., TSAO D., KIM Y.S. Effect of sodium butyrate on alkaline phosphatase in HRT-18, a human rectal cancer cell line. *Cancer Res.*1982;42:4540-4545.

330 GUM J.R., KAM W.K., BYRD J.C., HICKS J.W., SLEISINGER M.H., KIM Y.S. Effects of sodium butyrate on human colonic adenocarcinoma cells. Induction of placental-like alkaline phosphatase. *J. Biol. Chem.* 1987;262:1092-1097.

331 KIM Y.S., GUM J.R., HO S.B., DENG G. Colonocyte differentiation and proliferation: overview and the butyrate-induced transcriptional regulation of oncodevelopmental placental-like alkaline phosphatase gene in colon cancer cells. In: BINDER H.J., CUMMINGS J.H., SOERGEL K.H., eds. *Short-Chain Fatty Acids*. Lancaster, UK: Kluwer Academic, 1994: 119-134. (Falk Symposium; 73).

332 TOSCANI A., SOPRANO D.R., SOPRANO K.J. Sodium butyrate in combination with insulin or dexamethasone can terminally differentiate actively proliferating Swiss 3T3 cells into adipocytes. *J. Biol. Chem.* 1990;265:5722-5780.

333 LEDER A., LEDER P. Butyric acid, a potent inducer of erythroid differentiation in cultural erythroleukemic cells. *Cell* 1975;5:319-322.

334 GLADHAUG I.P., CHRISTOFFERSEN T. n-Butyrate and dexamethasone synergistically modulate the surface expression of epidermal growth factor receptors in cultured rat hepatocytes. *FEBS Lett.* 1989;243:21-24.

335 GLADHAUG I.P., REFSNES M., CHRISTOFFERSEN T. Regulation of hepatocyte epidermal growth factor receptors by n-butyrate and dimethyl sulfoxide:sensitivity to modulation by the tumor promoter TPA. *Anticancer Res.* 1989;9:1587-1592.

336 GLADHAUG I.P., REFSNES M., SAND T.-E., CHRISTOFFERSEN T. Effects of butyrate on epidermal growth factor receptor binding, morphology, and DNA synthesis in cultured rat hepatocytes. *Cancer Res.* 1988;48:6560-6564.

337 KOOISTRA T., VAN DEN BERG J., TONS A., PLATENBURG G., RIJKEN D.C., VAN DEN BERG E. Butyrate stimulates tissue-type plasminogen-activator synthesis in cultured human endothelial cells. *Biochem. J.* 1987;247:605-612.

338 CATTINI P.A., KARDAMI E., EBERHARDT N.L. Effect of butyrate on thyroid hormone-mediated gene expression in rat pituitary tumour cells. *Mol. Cell. Endocrin.* 1988;56:263-270.

339 BIRREN B.W., HERSCHMAN H.R. Regulation of the rat metallothionen-I gene by sodium butyrate. *Nucleic Acids Re*s. 1986;14:853-867.

340 ORMANDY C.J., DE FAZIO A., KELLY P.A., SUTHERLAND R.L. Coordinate regulation of oestrogen and prolactin receptor expression by sodium butyrate in human breast cancer cells. *Biochem. Biophys. Res. Commun.* 1992;182:740-745.

341 DE FAZIO A., CHIEW Y.-E., DONOGHUE C., LEE C.S.L., SUTHERLAND R.L. Effect of sodium butyrate on estrogen receptor and epidermal growth factor receptor gene expression in human breast cancer cell lines. *J. Biol. Chem.* 1992; 267:18008-18012.

342 SOULEIMANI A., ASSELIN C. Regulation of c-fos expression by sodium butyrate in the human colon carcinoma cell line Caco-2. *Biochem. Biophys. Res. Commun.* 1993;193:330-336.

343 Wice B.M., Trugnan G., Pinto M., *et al.* The intracellular accumulation of UDP-N-acetyl hexosamines is concomitant with the inability of human colon cancer cells to differentiate. *J. Biol. Chem.* 1985;260:139-146.

344 SAINI K., STEELE G., THOMAS P. Induction of carcinoembryonic-antigen-gene expression in human colorectal carcinoma by sodium butyrate. *Biochem. J.* 1990;272:541-544.

345 GIBSON P.R., ROSELLA O., ROSELLA G., YOUNG G.P. Butyrate is a potent inhibitor of urokinase secretion by colonic epithelium in vitro. *Gastroenterology* 1994;107:410-419.

346 GIBSON P.R., NOV R., FIELDING M., MCINTYRE A., ROSELLA O., MARIADASON J.M., FINCH C.F., BARKLA D.H., YOUNG G.P. Dietary modulation of urokinase and mucosal hydrolase activities. *Gastroenterology* 1996;110:A915.

347 BATES S.E., CURRIER S.J., ALVAREZ M., FOJO A.T. Modulation of P-Glycoprotein Phosphorylation and drug transport by sodium butyrate. *Biochemistry* 1992;31:6366-6372.

348 HIGGINS P.J., RYAN M.P. Cytoarchitecture of ras oncogene-expressing tumor cells: butyrate modulation of substrate adhesion, cytoskeletal actin content and subcellular microfilament distribution. *Int. J. Biochem.* 1989;21:1143-1151.

349 MALIK H., NORDENBERG J., NOVOGRODSKY A., FUCHS A., MALIK Z. Chemical inducers of differentiation, dimethylsulfoxide, butyric acid and dimethylthiourea, induce selective ultrasctructural patterns in B16 melanoma cells. *Biol. Cell* 1987;60: 33-40.

350 WILSON J.R., WEISER M.M. Colonic cancer cell (HT29) adhesion to laminin is altered by differentiation: adhesion may involve galactosyltransferase. *Exp. Cell Res.* 1992;201:330-334.

351 KRUH J., DEFER N., TICHONICKY L. Effects of butyrate on cell proliferation and gene expression. In: CUMMINGS J.H., ROMBEAU J.L., SAKATA T., eds. *Physiological and Clinical Aspects of Short-Chain Fatty Acids*. Cambridge, UK: Cambridge University Press, 1995: 275-288.

352 BOFFA L.C., LUPTON J.R., MARIANI M.R. *et al.* Modulation of colonic epithelial cell proliferation, histone acetylation, and luminal short chain fatty acids by variation of dietary fiber (wheat bran) in rats. *Cancer Res.* 1992;52:5906-5912.

353 JOHNSTON L.A., TAPSCOTT S.J., EISEN H. Sodium butyrate inhibits myogenesis by interfering with the transcriptional activation function of MyoD and Myogenin. *Mol. Cell. Biol*. 1992;12:5123-5130.

354 KLEHR D., SCHLAKE T., MAASS K., BODE J. Scaffold-attached regions (SAR Elements) mediate transcriptional effects due to butyrate. *Biochemistry* 1992;31:3222-3229.

355 BUSTIN S.A., NIE X.F., BARNARD R.C. Cloning and characterisation of ERF-1, a member of the Tis11 family of early-response genes. *DNA Cell Biol.* 1994;13:449-459.

356 MACLEAN K.N., SEE C.G., MCKAY I.A., BUSTIN S.A. The human immediate-early gene BRF1 maps to chromosome 14Q22-Q24. *Genomics* 1995;30(1):89-90.

357 HAGUE A., ELDER D.J.E., HICKS D.J., PARASKEVA C. Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. *Int. J. Cancer* 1995;60:400-406.

358 YOUNG G.P., GIBSON P.R. Butyrate and the human cancer cell. In: Cummings J.H., Rombeau J.L., Sakata T., eds. *Physiological and Clinical Aspects of Short-chain Fatty Acids*. Cambridge, UK: Cambridge University Press, 1995: 319-336.

359 CUMMINGS J.H., STEPHEN A.M., BRANCH W.J. Implications of dietary fiber breakdown in the human colon. In: BRUCE W.R., CORREA P., LIPKIN M., TANNENBAUM S.R., WILKINS T.D., eds. *Gastrointestinal Cancer: Endogenous Factors*. Cold Spring Harbor Laboratory, 1981: 71-81. (Banbury Report; 7).

360 AKANJI O.A., HOCKADAY T.D.R. Acetate tolerance and the kinetics of acetate utilization in diabetic and nondiabetic subjects. *Am. J. Clin. Nutr.* 1992;51:112-118.

361 LUNDQUIST F., SESTOFT L., DAMGAARD S.E., CLAUSEN J.P., TRAP-JENSEN J. Utilization of acetate in the human forearm during exercise after ethanol ingestion. *J. Clin. Invest.* 1973;52:3231-3235.

362 LINDENEG O., MELLEMGAARD K., FABRICIUS J., LUNDQUIST F. Myocardial utilization of acetate, lactate and free fatty acids after ingestion of ethanol. *Clin. Sci.* 1964;27:427-435.

363 WILLIAMSON J.R. Effects of insulin and starvation on the metabolism of acetate and pyruvate by the perfused rat heart. *Biochem. J.* 1964;93:97-106.

364 JUHLEN-DANNFELT A. Ethanol effects of substrate utilization by the human brain. *Scand. J. Clin. Lab. Invest.* 1977;37:443-449.

365 BUCKLEY B.M., WILLIAMSON D.H. Origins of blood acetate in the rat. *Biochem. J.* 1977;166:539-545.

366 SEUFERT C.D., GRAF M., JANSON G., KUHN A., SOLING H.D. Formation of free acetate by isolated perfused livers from normal, starved and diabetic rats. *Biochem. Biophys. Res. Commun.* 1974;57:901-909.

367 CROUSE J.R., GERSON C.D., DE CARLI L., LIEBER C.S. Role of acetate in the reduction of plasma free fatty acids produced by ethanol in man. *J. Lipid Res.* 1968;9:509-512.

368 BOUCHIER I.A.D., DAWSON A.M. The effect of infusions of ethanol on the plasma free fatty acids in man. *Clin. Sci.* 1964;26:47-54.

369 SCHEPPACH W., CUMMINGS J.H., BRANCH W.J., SCHREZENMEIR J. Effect of gut-derived acetate on oral glucose tolerance in man. *Clin. Sci.* 1988;75:355-361.

370 SCHEPPACH W., WIGGINS H.S., HALLIDAY D. *et al.* Effect of gut-derived acetate on glucose turnover in man. *Clin. Sci.* 1988;75:363-370.

371 BERGMAN D.N., ROE W.E., KON K. Quantitative aspects of propionate metabolism and gluconeogenesis in sheep. *Am. J. Physiol.* 1966;211:793-799.

372 Editorial. Propionate and cholesterol homeostasis in animals. *Nutr. Rev.* 1987;45:188-190.

373 CHEN W.-J.L., ANDERSON J.W., JENNINGS D. Propionate may mediate the hypocholesterolemic effects of certain soluble plant fibres in cholesterol-fed rats. P*roc. Soc. Exp. Biol. Med.* 1984;175:215-218.

374 ILLMAN R.J., TOPPING D.L., MCINTOSH G.H. *et al.* Hypocholesterolaemic effects of dietary propionate studies in whole animals and perfused rat liver. *Ann. Nutr. Metabol.* 1988;32:97-107.

375 BOILA R.J., SALOMONS M.D., MILLIGAN L.P., AHERNE F.X. The effect of dietary propionic acid on cholesterol synthesis in swine. *Nutr. Rep. Int.* 1981;23:1113-1121.

376 THACKER P.A., BOWLAND J.P. Effects of dietary propionic acid on serum lipids and lipoproteins of pigs fed diets supplemented with soybean meal or canola meal. *Can. J. Anim. Sci.* 1981;61:439-448.

377 THACKER P.A., SALOMONS M.O., AHERNE F.X., MILLIGAN L.P., BOWLAND J.P. Influence of propionic acid on the cholesterol metabolism of pigs fed hypercholesterolemic diets. *Can. J. Anim. Sci.* 1981;61:969-975.

378 THACKER P.A., BOWLAND J.P., FENTON M. Effects of vitamin B12 on serum lipids and lipoproteins of pigs fed diets supplemented with propionic acid or calcium propionate. *Can. J. Anim. Sci.* 1982;62:527-536.

379 VENTER C.S., VORSTER H.H., CUMMINGS J.H. Effects of dietary propionate on carbohydrate and lipid metabolism in man. *Am. J. Gastroenterol.* 1989;85:549-553.

380 TODESCO T., RAO V.A., BOSELLO O., JENKINS D.J.A. Propionate lowers blood glucose and alters lipid metabolism in healthy subjects. *Am. J. Clin. Nutr.* 1991;54:860-865.

381 STEPHEN A. Propionate-sources and effect on lipid metabolism. In: BINDER H.J., CUMMINGS J., SOERGEL K., eds. *Short-Chain Fatty Acids.* Lancaster, UK: Kluwer Academic Publishers, 1994: 260-271. (Falk Symposium; 73).

382 WOLEVER T.M.S., BRIGHENTI F., ROYALL D., JENKINS A.L., JENKINS D.J.A. Effect of rectal infusion of short chain fatty acids in human subjects. *Am. J. Gastroenterol.* 1989;84:1027-1033.

383 WOLEVER T.M.S., SPADAFORA P., ESHUIS H. Interaction between colonic acetate and propionate in humans. *Am. J. Clin. Nutr.* 1991;53:681-687.

384 ANDERSON J.W., BRIDGES S.R. Plant fiber metabolites alter hepatic glucose and lipid metabolism. *Diabetes* 1981;30(Suppl.1):133A.

385 REMESY C., DEMIGNE C., MORAND C. Metabolism of short-chain fatty acids in the liver. In: CUMMINGS J.H., ROMBEAU J.L., SAKATA T., eds. *Physiological and Clinical Aspects of Short-Chain Fatty Acids*. Cambridge, UK: Cambridge University Press, 1994:171-190.

386 LEVITT M.D. Volume and composition of human intestinal gas determined by means of an intestinal washout technic. *New Engl. J. Med.* 1971;284:1394-1398.

387 KIRK E. The quantity and composition of human colonic flatus. *Gastroenterology* 1949;12:782-794.

388 CALLOWAY D.H. Respiratory hydrogen and methane as affected by consumption of gas-forming foods. *Gastroenterology* 1966;51:383-389.

389 STEGGERDA F.R. Gastrointestinal gas following food consumption. *Ann. New York Acad. Sci.* 1968;150:57-66.

390 FLEMING S.E., CALLOWAY D.H., Determination of intestinal gas excretion. In: BIRCH G.G., PARKER K., eds. *Dietary fibre*. London: Applied Science Publishers, 1983:221-254.

391 MARTHINSEN D., FLEMING S.E. Excretion of breath and flatus gases by humans consuming high-fiber diets. *J. Nutr.* 1982;112:1133-1143.

392 CHRISTL S.U., MURGATROYD P.R., GIBSON G.R., CUMMINGS J.H. Production, metabolism and excretion of hydrogen in the large intestine. *Gastroenterology* 1992;102:1269-1277.

393 STEGGERDA F.R., RICHARDS E.A., RACKIS J.J. Effects of various soybean products on flatulence in the adult man. *Proc. Soc. Exp. Biol. Med.* 1966;121:1235-1239.

394 HICKEY C.A., CALLOWAY D.H., MURPHY E.L. Intestinal gas production following ingestion of fruits and fruit juices. *Am. J. Dig. Dis.* 1972;17:383-389.

395 HICKEY C.A., MURPHY E.L., CALLOWAY D.H. Intestinal gas production following ingestion of commercial wheaat cereals and milling fractions. *Cereal Chem.* 1972;49:276-282.

396 MEYER S., CALLOWAY D.H. Gastrointestinal response to oat and wheat milling fractions in older women. *Cereal Chem.* 1977;54:110-119.

397 BOND J.H., LEVITT M.D. Effect of dietary fiber on intestinal gas production and small bowel transit time in man. *Am. J. Clin. Nutr.* 1978;31:S169-S174.

398 MUIR J.G., LU Z.X., YOUNG G.P., CAMERON-SMITH D., COLLIER G.R., O'DEA K. Resistant starch in the diet increases breath hydrogen and serum acetate in human subjects. *Am. J. Clin. Nutr.* 1995;61:792-799.

399 TADESSE K., EASTWOOD M.A. Metabolism of dietary fibre components in man assessed by breath hydrogen and methane. *Br. J. Nutr.* 1978;40:393-396.

400 MCKAY L., BRYDON W.G., EASTWOOD M.A., SMITH J.H. The influence of pentose on breath methane. *Am. J. Clin. Nutr.* 1981;34:2728-2733.

401 FRITZ M., SIEBERT G., KASPER H. Dose dependence of breath hydrogen and methane in healthy volunteers after ingestion of a commercial disaccharide mixture, Palatinit. *Br. J. Nutr.* 1985;54:389-400.

402 FRITZ M., KASPER H., SCHREZENMEIR J., SIEBERT G. Effect of acarbose on the production of hydrogen and methane and on hormonal parameters in young adults under standardized low-fibre mixed diets. *Z. Ernahrungswiss.* 1985;24:1-18.

403 LEVITT M.D., LASSER R.B., SCHWARTZ J.S., BOND J.H. Studies of a flatulent patient. *New Engl. J. Med.* 1976;295:260-262.

404 LEVITT M.D., INGELFINGER F.J. Hydrogen and methane production in man. *Ann. New York Acad. Sci.* 1968;150:75-81.

405 LEVITT M.D. Production and excretion of hydrogen gas in man. *New Engl. J. Med.* 1969;281:122-127.

406 CALLOWAY D.H., COLASITO D.J., MATHEWS R.D. Gases produced by human intestinal microflora. *Nature* 1966; 212:1238-1239.

407 BOND J.H., ENGEL R.R., LEVITT M.D. Factors influencing pulmonary methane excretion in man. An indirect method of studying the in situ metabolism of the methane-producing colonic bacteria. *J. Exp. Med.* 1971;133:572-588.

408 CHEN S., ZIEVE L., MAHADEVAN V. Mercaptans and dimethyl sulfide in the breath of patients with cirrhosis of the liver. *J. Lab. Clin. Med.* 1970;75:628-635.

409 ROBIN E.D., TRAVIS D.M., BROMBERG P.A., FORKNER C.E., TYLER J.M. Ammonia excretion by mammalian lung. *Science* 1959;129:270-271.

410 WOLIN M.M. The rumen fermentation: a model for microbial interactions in anaerobic ecosystems. *Adv. Microb. Ecol.* 1979;3:49-77.

411 BOND J.H., LEVITT M.D. Use of pulmonary hydrogen (H2) measurements to quantitate carbohydrate absorption. Study of partially gastrectomized patients. *J. Clin. Invest.* 1972;51:1219-1225.

412 CALLOWAY D.H., MURPHY E.L., BAUER D. Determination of lactose intolerance by breath analysis. *Am. J. Dig. Dis.* 1969;14:811-815.

413 LEVITT M.D., DONALDSON R.M. Use of respiratory hydrogen (H2) excretion to detect carbohydrate malabsorption. *J. Lab. Clin. Med.* 1970;75:937-945.

414 METZ G., JENKINS D.J.A., PETERS T.J., NEWMAN A., BLENDIS L.M. Breath hydrogen as a diagnostic method for hypolactasia. *Lancet* 1975;i:1155-1156.

415 MILLER R.S., HOSKINS L.C. Mucin degradation in human colon ecosystems. Fecal population densities of mucin-degrading bacteria estimated by a “most probable number” method. *J. Clin. Invest* 1981;67:759-765.

416 MACFARLANE G.T., HAY S., MACFARLANE S., GIBSON G.R. Effect of different carbohydrates on growth, polysaccharidase and glycosidase production by *Bacteroides ovatus*, in batch and continuous culture. *J. Appl. Bacteriol.* 1990;68:179-187.

417 DEGNAN B.A., MACFARLANE G.T. Comparison of carbohydrate substrate preferences in eight species of bifidobacteria. FEMS *Microbiol. Let.* 1991;84:151-156.

418 BOND J.H., LEVITT M.D. Investigation of small bowel transit time in man utilising pulmonary H measurements. *J. Lab. Clin. Med.* 1974;85:546-559.

419 BROOY L.S.J., MALE P.J., BEAVIS A.K., MISIEWICZ J.J. Assessment of the reproducibility of the lactulose H2 breath test as a measure of mouth to caecum transit time. *Gut* 1983;24:893-896.

420 READ N.W., MILES C.A., FISHER D. Transit of a meal through the stomach, small intestine, and colon in normal subjects and its role in the pathogenesis of diarrhoea. *Gastroenterology* 1980;79:1276-1282.

421 READ N.W., AL-JANABI M.N., BATES T.E. Interpretation of the breath hydrogen profile obtained after ingesting a solid meal containing unabsorbable carbohydrate. *Gut* 1985;26:834-842.

422 BJORNEKLETT A., JENSSEN E. Relationships between hydrogen (H2) and methane (CH4) production in man. *Scand. J. Gastroenterol.* 1982;17:985-992.

423 ROBB T.A., GOODWIN D.A., DAVIDSON G.P. Faecal hydrogen production in vitro as an indicator for in vivo hydrogen producing capability in the breath hydrogen test. *Acta Paediatr. Scand.* 1985;74:942-944.

424 PERMAN J.A., MODLER S., OLSON A.C. Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora. *J. Clin. Invest.* 1981;67:643-650.

425 PIQUE J.M., PALLARES M., CUSO E., VILAR-BONET J., GASSULL M.A. Methane production and colon cancer. *Gastroenterology* 1984;87:601-605.

426 HAINES A., METZ G., DILAWARI J., BLENDIS L., WIGGINS H. Breath methane in patients with cancer of the large bowel. *Lancet* 1977;ii:481-483.

427 MCKAY L.F., EASTWOOD M.A., BRYDON W.G. Methane excretion in man: a study of breath, flatus and faeces. *Gut* 1985;26:69-74.

428 PITT P., DE BRUIJN K.M., BEECHING M.F., GOLDBERG E., BLENDIS L.M. Studies on breath methane: the effect of ethnic origins and lactulose. *Gut* 1980;21:951-959.

429 DRASAR B.S., TOMKINS A.M., WIGGINS H., HUDSON M. Breath methane levels and intestinal methanogenesis among rural Nigerians on a local diet. *Proc. Nutr. Soc.* 1984;43:86A.

430 PELED Y., GILAT T., LIBERMAN E., BUJANOVER Y. The development of methane production in childhood and adolescence. *J. Pediatr. Gastroenterol. Nutr.* 1985;4:575-579.

431 HOFF G., BJORNEKLETT A., MOEN I.E., JENSSEN E. Epidemiology of polyps in the rectum and sigmoid colon. Evaluation of breath methane and predisposition for colorectal neoplasia. *Scand. J. Gastroenterol.* 1986;21:193-198.

432 KARLIN D.A., MASTROMARINO A.J., JONES R.D., STROEHLEIN J.R., LORENTZ O. Fecal skatole and indole and breath methane and hydrogen in patients with large bowel polyps or cancer. *J. Cancer Res. Clin. Oncol.* 1985;109:135-141.

433 MILLER T.L., WOLIN M.J. Enumeration of *Methanobrevibacter smithii* in human feces. *Arch. Microbiol.* 1982;131:14-18.

434 MILLER T.L., WOLIN M.J. *Methanosphaera stadtmaniae* gen. nov., sp. nov.: a species that forms methane by reducing methanol with hydrogen. *Arch. Microbiol.* 1985;141:116-122.

435 MILLER T.L., WOLIN M.J. Methanogens in human and animal intestinal tracts. *Syst. Appl. Microbiol.* 1986;7:223-229.

436 MILLER T.L., WOLIN M.J. Oxidation of hydrogen and reduction of methanol to methane is the sole energy source for a methanogen isolated from human feces. *J. Bacteriol.* 1983;153:1051-1055.

437 WOLIN M.J. Metabolic interactions among intestinal microorganisma. *Am. J. Clin. Nutr.* 1974;27:1320-1328.

438 MACFARLANE G.T., CUMMINGS J.H. The colonic flora, fermentation and large bowel digestive function. In: PHILLIPS S.F., PEMBERTON J.H., SHORTER R.G., eds. *The Large Intestine: Physiology, Pathophysiology and Diseases*. New York: Raven Press, 1990:51-92.

439 FLOURIE B., FLORENT C., JOUANY J.P., THIVEND P., ETANCHAUD F., RAMBAUD J.C. Colonic metabolism of wheat starch in healthy humans. Effects on fecal outputs and clinical symptoms. *Gastroenterology* 1986;90:111-119.

440 BEERENS H., ROMOND C. Sulfate-reducing anaerobic bacteria in human feces. *Am. J. Clin. Nutr.* 1977;30:1770-1776.

441 GIBSON G.R., MACFARLANE S., MACFARLANE G.T. Metabolic interactions involving sulphate-reducing and methanogenic bacteria in the human large intestine. *FEMS Microbiol. Ecol.* 1993;12:117-125.

442 GIBSON G.R., CUMMINGS J.H., MACFARLANE G.T. Growth and activities of sulphate-reducing bacteria in gut contents from healthy subjects and patients with ulcerative colitis. *FEMS Microbiol. Ecol.* 1991;86:103-112.

443 GIBSON G.R., CUMMINGS J.H., MACFARLANE G.T. Use of a three-stage continuous culture system to study the effect of mucin on dissimilatory sulfate reduction and methanogenesis by mixed populations of human gut bacteria. *Appl. Environ. Microbiol.* 1988;54:2750-2755.

444 GIBSON G.R. A review - The physiology and ecology of sulphate-reducing bacteria. *J. Appl. Bacteriol.*1990;69:769-797.

445 GIBSON G.R., MACFARLANE G.T., CUMMINGS J.H. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. *J. Appl. Bacteriol.*1988;65:103-111.

446 CHRISTL S.U., GIBSON G.R., CUMMINGS J.H. Methanogenesis in the human large intestine. *Gut* 1993;34:573-574.

447 CHRISTL S.U., GIBSON G.R., CUMMINGS J.H. Role of dietary sulphate in the regulation of methanogenesis in the human large intestine. *Gut* 1992;33:1234-1238.

448 GIBSON G.R., CUMMINGS J.H., MACFARLANE G.T. *et al.* Alternative pathways for hydrogen disposal during fermentation in the human colon. *Gut* 1990; 31:679-683.

449 KRISTJANSSON J.K., SCHONHEIT P., THAUER R.K. Different Ks values for hydrogen of methanogenic bacteria and sulfate-reducing bacteria: an explanation for the aparent inhibition of methanogenesis by sulfate. *Arch. Microbiol.* 1982;131:278-282.

450 FLORIN T.H.J., NEALE G., GIBSON G.R., CHRISTL S.U., CUMMINGS J.H. Metabolism of dietary sulphate: absorption and excretion in humans. *Gut* 1991;32:766-773.

451 FLORIN T.H.J., NEALE G., GORETSKI S., CUMMINGS J.H. The sulphate content of foods and beverages. *J. Food Compos. Anal.* 1993;6:140-151.

452 MACFARLANE G.T., GIBSON G.R. Formation of glycoprotein degrading enzymes by Bacteroides fragilis. *FEMS Microbiol. Let.* 1991;77:289-294.

453 MACFARLANE G.T., HAY S., GIBSON G.R. Influence of mucin on glycosidase, protease and arylamidase activities of human gut bacteria grown in a 3-stage continuous culture system. *J. Appl. Bacteriol.* 1989;66:407-417.

454 TSAI H.H., SUNDERLAND D., GIBSON G.R., HART C.A., RHODES J.M. A novel mucin sulphatase from human faeces: its identification, purification and characterization. *Clin. Sci.* 1992;82:447-454.

455 VERCELLOTTI J.R., SALYERS A.A., BULLARD W.S., WILKINS T.D. Breakdown of mucin and plant polysaccharides in the human colon. *Can. J. Biochem.* 1977;55:1190-1196.

456 SMITH L., KRYSZYNA H., SMITH R.P. The effect of methaemoglobin on the inhibition of cytochrome c oxidase by cyanide, sulfide or azide. *Biochem. Pharmacol.* 1977;22:47-50.

457 FLORIN T.H.J., NEALE G., CUMMINGS J.H. The effect of hydrogen sulphide on isolated rat colonic epithelium. In : *Advances in the Treatment of Ulcerative Colitis* (abstracts): 1990: 26 (Falk Symposium; 60).

458 LAJOIE S.F., BANKS S., MILLER T.L., WOLIN M.J. Acetate production from hydrogen and (^{13}C) carbon dioxide by the microflora of human feces. *Appl. Environ. Microbiol.* 1988;54:2723-2727.

459 DURAND M., BERNALIER A. Reductive acetogenesis in animal and human gut. In: CUMMINGS J.H., ROMBEAU J.L., SAKATA T., eds. *Physiological and Clinical Aspects of Short-Chain Fatty Acids.* Cambridge, UK: Cambridge University Press, 1995: 107-117.

460 CHRISTL S.U., GIBSON G.R., MURGATROYD P.R., SCHEPPACH W., CUMMINGS J.H. Impaired hydrogen metabolism in pneumatosis cystoides intestinalis. *Gastroenterology* 1993;104:392-397.

461 ECKER J.A., WILLIAMS R.G., CLAY K.L. Pneumatosis cystoides intestinalis-bullous emphysema of the intestine. *Am. J. Gastroenterol.* 1971;56:125-136.

462 FORGACS P., WRIGHT P.H., WYATT A.P. Treatment of intestinalis gas cysts by oxygen breathing. *Lancet* 1973;1:579-582.

463 GILLON T., TADESSE K., LOGAN R.F.A., HOLT S., SIRCUS W. Breath hydrogen in pneumatosis cystoides intestinalis. *Gut* 1979;20:1008-1011.

464 READ N.W., AL-JANABI M.N., CANN P.A. Is raised breath hydrogen related to the pathogenesis of pneumatosis coli? *Gut* 1984;25:839-845.

465 FLORIN T.H.J., HILLS B.A. Does counterperfusion supersaturation cause gas cysts in pneumatosis cystoides coli, and can breathing heliox reduce them? *Lancet* 1995;345:1220-1222.

466 LEVITT M.D., OLSSON S. Pneumatosis cystoides intestinalis and high breath H2 excretion: insights into the role of H2 in this condition. *Gastroenterology* 1995;108:1560-1565.

467 ROEDIGER W.E.W. The colonic epithelium in ulcerative colitis: an energy-deficiency disease? *Lancet* 1980;2:712-715.

468 IRELAND A., JEWELL D.P. 5-aminosalicylic acid (5-ASA) has no effect on butyrate metabolism in human colonic epithelial cells. *Gastroenterology* 1989;98:A176.

469 CHAPMAN M.A.S., GRAHN M., GIAMUNDO P., O'CONNELL P.R., ONWU D., HUTTON M. *et al.* A new technique to measure mucosal metabolism and its use to map substrate utilisation in the healthy human large bowel. *Br. J. Surg.* 1993;80:445-449.

470 CHAPMAN M.A.S., GRAHN M.F., BOYLE M.A., HUTTON M., ROGERS J., WILLIAMS N.S. Butyrate oxidation is impaired in the colonic mucosa of sufferers of quiescent ulcerative colitis. *Gut* 1994;35:73-76.

471 WILLIAMS N.N., BRANIGAN A., FITZPATRICK J.M., O'CONNELL P.R. Glutamine and butyric acid metabolism measurement in biopsy specimens (in vivo): a method of assessing treatment on inflammatory bowel conditions. *Gastroenterology* 1992;102:A713.

472 FINNIE I.A., TAYLOR B.A., RHODES J.M. Ileal and colonic epithelial metabolism in quiescent ulcerative colitis: increased glutamine metabolism in distal colon but no defect in butyrate metabolism. *Gut* 1994;34:1552-1558.

473 ROEDIGER W.E.W. Colonic epithelial metabolism in ulcerative colitis. *Gut* 1993;34:1646.

474 ROEDIGER W.E.W., LAWSON M.J., KWOK V., KERR GRANT A., PANNALL P.R. Colonic bicarbonate output as a test of disease activity in ulcerative colitis. *J. Clin. Pathol.* 1984;37:704-707.

475 ROEDIGER W.E.W., NANCE S. Metabolic induction of experimental ulcerative colitis by inhibition of fatty acid oxidation. *Br. J. Exp. Pathol.* 1986;67:773-782.

476 MARCUS R., WATT J. Seaweeds and ulcerative colitis in laboratory animals. *Lancet* 1969;August:489-490. (Letters).

477 MARCUS R., WATT J., PATH M.R.C. Ulcerative disease of the colon in laboratory animals induced by pepsin inhibitors. *Gastroenterology* 1974;67:473-483.

478 ONDERDONK A., HERMOS J., BARTLETT J. The role of the intestinal microflora in experimental colitis. *Am. J. Clin. Nutr.* 1977;30:1819-1825.

479 VAN DE WAAIJ D., COHEN B.J., ANVER M.R. Mitigation of experimental inflammatory bowel disease in guinea pigs by selective elimination of the aerobic gram-negative intestinal microflora. *Gastroenterology* 1974;67:460-472.

480 ONDERDONK A.B., HERMOS J.A., DZINK J.L., BARTLETT J.G. Protective effect of metronidazole in experimental ulcerative colitis. *Gastroenterology* 1978; 74:521-526.

481 KITANO A., KOBAYASHI K., OSHIUMI H. *et al*. Studies of experimental ulcerative colitis induced by carrageenan in rabbits. *Jpn. J. Gastroenterol.* 1981;78:2104-2111.

482 ONDERDONK A.B., BARTLETT M.D. Bacteriological studies of experimental ulcerative colitis. *Am. J. Clin. Nutr.* 1979;32:258-265.

483 HIRONO I., SUMI Y., KUHARA K., MIYAKAWA M. Effect of degraded Carrageenan on the intestine in germfree rats. *Toxicol. Let.* 1981;8:207-212.

484 OHKUSA T. Production of experimental ulcerative colitis in hamsters by dextran sulfate sodium and change in intestinal microflora. *Jpn. J. Gastroenterol.* 1985;82:1327-1336.

485 OKAYASU I., HATAKEYAMA S., YAMADA M., OHKUSA T., INAGAKI Y., NAKAYA R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 1990;98:694-702.

486 OHKUSA T., YAMADA M., TAKENAGA TEA. Protective effect of metronidazole in experimental ulcerative colitis induced by dextran sulfate sodium. *Jpn. J. Gastroenterol.* 1987;84:2337-2346.

487 PITCHER M.C.L., BEATTY E.R., GIBSON G.R., CUMMINGS J.H. Incidence and activities of sulphate-reducing bacteria in patients with ulcerative colitis. *Gut* 1995;36:A63.

488 AXELSSON L.G., LUNDSTROM L., BYLUND-FELLENIUS A.C., MIDTVEDT T. Dextran sulphate sodium induced colitis and sulfasalazine treatment in bacteria-free mice. *Gastroenterology* 1995;108:A775.

489 AXELSSON L.-G., LANDSTROM E., BYLUND-FELLENIUS A.C. Sulfasalazine and olsalazine are effective for the treatment of dextran sulfate induced colitis in athymic mice. *Gastroenterology* 1994;106:A648.

490 BYLUND-FELLENIUS A.C., LANDSTROM E., AXELSSON L.-G. Sulfasalazine ameliorates colitis induced by oral administration of dextran sulfate in mice. *Gastroenterology* 1994;106:A659.

491 CHEN Y., CONNER E.M., GRISHAM M.B. Dextran sulfate sodium induces colitis in rats by enhancing mucosal permeability: effects of sulfasalazine. *Gastroenterology* 1995;108:A796.

492 YOSHIZUMI M., HIRATA I., SASAKI SEA. Effects of various drugs on chronic colitis induced by dextran sulfate sodium in rats. *Gastroenterology* 1995;108:A947.

493 PITCHER M.C.L., BEATTY E.R., CUMMINGS J.H. Salicylates inhibit bacterial sulphide production within the colonic lumen in ulcerative colitis. *Gut* 1995;37:A15.

494 ROEDIGER W.E.W., DUNCAN A., KAPANIRIS O., MILLARD S. Reducing sulfur compounds of the colon impair colonocyte nutrition: implications for ulcerative colitis. *Gastroenterology* 1993;104:802-809.

495 DUNCAN A., KAPANIRIS O., ROEDIGER W.E.W. Measurement of mercaptoacetate levels in anaerobic batch culture of colonic bacteria. *FEMS Microbiol. Ecol.* 1990;74:303-308.

496 ROEDIGER W.E.W., DUNCAN A., KAPANIRIS O., MILLARD S. Sulphide impairment of substrate oxidation in rat colonocytes: a biochemical basis for ulcerative colitis? *Clin. Sci.* 1993;85:623-627

497 MOORE J.W.E., ROEDIGER W.E.W. Hydrogen sulphide in vivo diminishes fatty acid oxidation in the colonic mucosa; implications for ulcerative colitis. In: *Inflammatory Bowel Disease* (abstracts), 1995:56 (Falk Symposium; 85).

498 ASLAM M., BATTEN J.J., FLORIN T.H.J., SIDEBOTHAM R.L., BARON J.H. Hydrogen sulphide induced damage to the colonic mucosal barrier in the rat. *Gut* 1992;33:F274.

499 MAYALL T., MACPHERSON A., BJARNASON I., FORGACS I., PETERS T. Mitochondrial function of colonic epithelial cells in inflammatory bowel disease. *Gut* 1992;33:S24.

500 STEIN J., SCHRÖDER O., MILOVIC V., CASPARY W. Mercaptopropionate inhibits butyrate uptake in isolated apical membrane vesicles of the rat distal colon. *Gastroenterology* 1995;108:673-679.

501 ROEDIGER W.E.W., HEYWORTH M., WILLOUGHBY P., PIRIS J., MOORE A., TRUELOVE S.C. Luminal ions and short chain fatty acids as markers of functional activity of the mucosa in ulcerative colitis. *J. Clin. Pathol.* 1982;35:323-326.

502 VERNIA P., GNAEDINGER A., HAUCK W., BREUER R.I. Organic anions and the diarrhea of inflammatory bowel disease. *Dig. Dis. Sci.* 1988;33:1353-1358.

503 VERNIA P., CAPRILLI R., LATELLA G., BARBETTI F., MAGLIOCCA F.M., CITTADINI M. Fecal lactate and ulcerative colitis. *Gastroenterology* 1988;95:1565-1568.

504 HOVE H., CLAUSEN M.R., MORTENSEN P.B. Lactate and pH in faeces from patients with colonic adenomas or cancer. *Gut* 1993;34:625-629.

505 BREUER R.I., BUTO S.K., CHRIST M.L. *et al.* Rectal irrigation with short chain fatty acids for distal ulcerative colitis. *Dig. Dis. Sci.* 1991;36:185-187.

506 BREUER R.I., CHRIST M.L., LASHNER B.A. *et al.* Short-chain fatty acid rectal irrigation therapy for left sided ulcerative colitis: a randomized, placebo-controlled clinical trial. In: BINDER H.J., CUMMINGS J., SOERGEL K.H., eds. *Short-Chain Fatty Acids.* Lancaster, UK: Kluwer Academic Publishers, 1994: 214-220. (Falk Symposium; 73).

507 SCHEPPACH W., SOMMER H., KIRCHNER T. *et al.* Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. *Gastroenterology* 1992;103:51-56.

508 SENAGORE A.J., MACKEIGAN J.M., SCHEIDER M., EBROM J.S. Short-chain fatty acid enemas: a cost effective alternative in the treatment of non-specific proctosigmoiditis. *Dis. Colon Rectum* 1992;35:923-927.

509 VERNIA P., CITTADINI M., MARCHEGGIANO A. *et al.* Short chain fatty acid (SCFA) enemas in distal ulcerative colitis: a double blind placebo controlled study. In: *Short-Chain Fatty Acids* (abstracts), 1993: D8 (Falk Symposium; 73).

510 VERNIA P., MARCHEGGIANO A., CAPRILLI R. *et al.* Short-chain fatty acid topical treatment in distal ulcerative colitis. *Aliment. Pharmacol. Ther.* 1995;9:309-313.

511 STEINHART A.H., BAKER J.P., BRZEZINSKI A. Butyrate enemas in the treatment of refractory ulcerative proctosigmoiditis. In: *Short-Chain Fatty Acids* (abstracts), 1993: D1 (Falk Symposium; 73).

512 STEINHART A.H., HIRUKI T., BRZEZINSKI A., BAKER J.P. Treatment of left-sided ulcerative colitis with butyrate enemas: a randomized controlled trial. *Gastroenterology* 1995;108:A921.

513 PITCHER M.C.L., BEATTY E.M., WARING R.H., CUMMINGS J.H. Is there a defect in sulphur metabolism in ulcerative colitis? *Gut* 1995;37:A22.

514 GLOTZER D.J., GLICK M.E., GOLDMAN H. Proctitis and colitis following diversion of the fecal stream. *Gastroenterology* 1981;80:438-441.

515 KORELITZ B.I., CHESKIN L.J., SOHN N., SOMMERS S.C. Proctitis after fecal diversion in Crohn's disease and its elimination with reanastomosis: implications for surgical management. *Gastroenterology* 1984;87:710-713.

516 NEUT C., COLOMBEL J.F., GUILLEMOT F. *et al.* Impaired bacterial flora in human excluded colon. *Gut* 1989;30:1094-1098.

517 LUSK L.B., REICHEN J., LEVINE J.S. Aphthous ulceration in diversion colitis. *Gastroenterology* 1984;87:1171-1173.

518 ROEDIGER W.E.W. The starved colon - diminished mucosal nutrition, diminished absorption and colitis. *Dis. Colon Rectum* 1990;33:858-862.

519 HARIG J.M., SOERGEL K.H., KOMOROWSKI R.A., WOOD C.M. Treatment of diversion colitis with short-chain fatty acid irrigation. *New Engl. J. Med.* 1989;320:23-28.

520 GUILLEMOT F., COLOMBEL J.F., NEUT C. *et al.* Treatment of diversion colitis by short chain fatty acids. *Dis. Colon Rectum* 1991;34:861-864.

521 WISCHMEYER P.E., TREMAINE W.J., HADDAD A.C., AMBROZE W.L., PEMBERTON J.H., PHILLIPS S.F. Fecal short chain fatty acids in patients with pouchitis after ileal pouch anal anastomosis. *Gastroenterology* 1991;100:A848.

522 CLAUSEN M.R., TVEDE M., MORTENSEN P.B. Short chain fatty acids in pouch contents from patients with and without pouchitis after ileal pouch-anal anastomosis. *Gastroenterology* 1992;103:1144-1153.

523 DE SILVA H.J., IRELAND A., KETTLEWELL M., MORTENSEN N., JEWELL D.P. Short chain fatty acid irrigation in severe pouchitis. *New Engl. J. Med.* 1989;321:1416-1417.

524 WISCHMEYER P., GROTZ R.L., PEMBERTON J.H., PHILLIPS S.F. Treatment of pouchitis after ileo-anal anastomosis with glutamine and butyric acid. *Gastroenterology* 1992;102:A617.

525 WEAVER L.T. Review: Bowel habit from birth to old age. *J. Pediatr. Gastroenterol. Nutr.* 1988;7:637-640.

526 RENDTORFF R.C., KASHGARIAN M. Stool patterns of healthy adult males. *Dis. Colon Rectum* 1967;10:222-228.

527 BASS P., DENNIS S. The laxative effects of lactulose in normal and constipated subjects. *J. Clin. Gastroenterol.* 1981;3:23-28.

528 THOMPSON W.G., HEATON K.W. Functional bowel disorders in apparently healthy people. *Gastroenterology* 1980;79:283-288.

529 DAVIES G.J., CROWDER M., REID B., DICKERSON J.W.T. Bowel function measurements of individuals with different eating patterns. *Gut* 1986;27:164-169.

530 GRAHAM D.Y., MOSER S.E., ESTES M.K. The effect of bran on bowel function in constipation. *Am. J. Gastroenterol.* 1982;77:599-603.

531 EASTWOOD M.A., BRYDON W.G., BAIRD J.D. *et al.* Fecal weight and composition, serum lipids and diet among subjects aged 18 to 80 not seeking health care. *Am. J. Clin. Nutr.* 1984;40:628-634.

532 WYMAN J.B., HEATON K.W., MANNING A.P., WICKS A.C.B. Variability of colonic function in healthy subjects. *Gut* 1978;19:146-150.

533 BINGHAM S.A., CUMMINGS J.H. Effect of exercise and physical fitness on large intestinal function. *Gastroenterology* 1989;97:1389-1399.

534 TUCKER D.M., SANDSTEAD H.H., LOGAN G.M. *et al.* Dietary fiber and personality factors as determinants of stool output. *Gastroenterology* 1981;81:879-883.

535 CUMMINGS J.H., JENKINS D.J.A, WIGGINS H.S. Measurement of the mean transit time of dietary residue through the human gut. *Gut* 1976;17:210-218.

536 HINTON J.M., LENNARD-JONES J.E., YOUNG A.C. A new method for studying gut transit times using radio-opaque markers. *Gut* 1969;10:842-847.

537 CASSANO C.C., DEL VECCHIO E., CAPURSO L. I tempi di transito gastrointestinali. *Radiol. Pract.* 1968;18:207-223.

538 EASTWOOD H.D.H. Bowel transit studies in the elderly: radio-opaque markers in the investigation of constipation. *Gerontol. Clin.* 1972;14:154-159.

539 HINTON J.M., LENNARD-JONES J.E. Constipation: definition and classification. *Postgrad. Med. J.* 1968;44:720-723.

540 ABRAHAMSON H., ANTOV S., BOSAEUS I. Gastrointestinal and colonic segmental transit time evaluated by a single abdominal x-ray in healthy subjects and constipated patients. *Scand. J. Gastroenterol.* 1988;23:72-80.

541 CHAUSSADE S., ROCHE H., KHYARI A., COUTURIER D., GUERRE J. Mesure du temps de transit colique (TTC): description et validation d'une nouvelle technique. *Gastroenterol. Clin. Biol.* 1986;10:385-389.

542 CHAUSSADE S., KHYARI A., ROCHE H. *et al.* Determination of total and segmental colonic transit time in constipated patients. Results in 91 patients with a new simplified method. *Dig. Dis. Sci.* 1989;34:1168-1172.

543 STASSE-WOLTHUIS M., KATAN M.B., HAUTVAST J.G.A.J. Fecal weight, transit time and recommendations for dietary fiber intake. *Am. J. Clin. Nutr.* 1978;31:909-910.

544 SPILLER G.A. Suggestions for a basis on which to determine a desirable intake of dietary fiber. In: SPILLER G.A., ed. *CRC Handbook of Dietary Fiber in Human Nutrition*. Boca Raton, Florida: CRC Press, 1986: 281-283.

545 CUMMINGS J.H. Constipation, dietary fibre and the control of large bowel function. *Postgrad. Med. J.* 1984;60:811-819.

546 CUMMINGS J.H., HILL M.J., JIVRAJ T., HOUSTON H., BRANCH W.J., JENKINS D.J.A. The effect of meat protein and dietary fiber on colonic function. Part I. Changes in bowel habit, bile acid excretion and calcium absorption. *Am. J. Clin. Nutr.* 1979;32:2086-2093.

547 CUMMINGS J.H., WIGGINS H.S., JENKINS D.J.A. *et al.* Influence of diets high and low in animal fat on bowel habit, gastrointestinal transit time, fecal microflora, bile acid and fat excretions. *J. Clin. Invest.* 1978;61:953-963.

548 Ministry of Agriculture Fisheries and Food. *Annual Report of the National Food Survey*. London: HMSO, 1990.

549 CUMMINGS J.H. Non-starch polysaccharides (dietary fibre) including bulk laxatives in constipation. In: KAMM M.A., LENNARD-JONES J.E., eds. *Constipation*. Petersfield, UK: Wrightson Biomedical Publishing, 1994: 307-314.

550 CUMMINGS J.H. The effect of dietary fiber on fecal weight and composition. In: SPILLER G.A., ed. *CRC Handbook of Dietary Fiber in Human Nutrition*. 2d ed. Boca Raton, Florida: CRC Press, 1993: 263-349.

551 TOMLIN J., READ N.W. The relation between bacterial degradation of viscous polysaccharides and stool output in human beings. *Br. J. Nutr.* 1988;60:467-475.

552 TOMLIN J., READ N.W. Laxative properties of indigestible plastic particles. *Br. Med. J.* 1988;297:1175-1176.

553 CUMMINGS J.H. Dietary fibre and the intestinal microflora. In: HALLGREN B., ed. *Nutrition and the Intestinal Flora*. Stockholm: Swedish Nutrition Foundation, 1983: 77-86.

554 KURPAD A.V., SHETTY P.S. Effects of antimicrobial therapy on faecal bulking. *Gut* 1986;27:55-58.

555 PRYNNE C.J., SOUTHGATE D.A.T. The effects of a supplement of dietary fibre on faecal excretion by human subjects. *Br. J. Nutr.* 1979;41:495-503.

556 CUMMINGS J.H., SOUTHGATE D.A.T., BRANCH W., HOUSTON H., JENKINS D.J.A., JAMES W.P.T. The colonic response to dietary fibre from carrot, cabbage, apple, bran and guar gum. *Lancet* 1978;i:5-9.

557 TOMLIN J., READ N.W. A comparative study of the effects on colon function caused by feeding ispaghula husk and polydextrose. *Aliment. Pharmacol. Ther.* 1988;2:513-519.

558 HESPELL R.B. Efficiency of growth by ruminal bacteria. *Fed. Proc.* 1979; 38:2707-2712.

559 HESPELL R.B., BRYANT M.P. Efficiency of rumen microbial growth: influence of some theoretical and experimental factors on ATP. *J. Anim. Sci.* 1979;49:1640-1659.

560 GREGORY J., FOSTER K., TYLER H. T., WISEMAN M. *The dietary and nutritional survey of British adults*. Office of Population Censuses and Surveys. London: HMSO, Publications Centre 1990.

561 KAMM M.A., LENNARD-JONES J.E., eds. *Constipation*. Petersfield, UK: Wrightson Biomedical Publishing, 1994.

562 CUMMINGS J.H. Constipation. In: MISIEWICZ J.J., POUNDER R.E., VENABLES C.W., eds. *Diseases of the Gut and Pancreas.* 2d ed. Oxford: Blackwell Scientific Publications, 1994: 51-70.

563 DEVROEDE G. Constipation. In: SLEISENGER M.H., FORDTRAN J.S., eds. *Gastrointestinal Disease: Pathophysiology, Diagnosis, Management.* 5th ed. Philadelphia, USA: W.B. Saunders, 1994: 837-887.

564 SANDLER R.S., DROSSMAN D.A. Bowel habits in young adults not seeking health care. *Dig. Dis. Sci.* 1987;32:841-845.

565 MOORE-GILLON V. Constipation: what does the patient mean? *J. R. Soc. Med.* 1984;77:108-110.

566 SANDLER R.S., JORDAN M.C., SHELTON B.J. Demographic and dietary determinants of constipation in the US population. *Am. J. Public Health* 1990;80:185-189.

567 FOTHERBY K.J., HUNTER J.O. Idiopathic slow-transit constipation: whole gut transit times, measured by a new simplified method, are not shortened by opioid antagonists. *Aliment. Pharmacol. Ther.* 1987;1:331-338.

568 PRESTON D.M., LENNARD-JONES J.E. Severe chronic constipation of young women: 'idiopathic slow transit constipation'. *Gut* 1986;27:41-48.

569 BARRETT J.A. Management of constipation. *Gen. Med.* Suppl.: *Constipation* 1989;(March):14-18.

570 ROE A.M., BARTOLO D.C.C., MORTENSEN N.J.M. Diagnosis and surgical management of intractable constipation. *Br. J. Surg.* 1986;73:854-861.

571 WALD A. Colonic transit and anorectal monometry in chronic idiopathic constipation. *Arch. Intern. Med.* 1986;146:1713-1716.

572 WATIER A., DEVROEDE G., DURANCEAU A. Constipation with colonic inertia: a manifestation of systemic disease? *Dig. Dis. Sci.* 1983;28:1025-1033.

573 RAO S.S.C., READ N.W., BROWN C., BRUCE C., HOLDSWORTH C.D. Studies on the mechanism of bowel disturbance in ulcerative colitis. *Gastroenterology* 1987; 93:934-940.

574 WEBER J., DENIS P., MIHOUT B., *et al.* Effect of brain-stem lesions on colonic and anorectal motility. Study of three patients. *Dig. Dis. Sci.* 1985;30:419-425.

575 MARTELLI H., DEVROEDE G., ARHAN P., DUGUAY C. Mechanisms of idiopathic constipation: outlet obstruction. *Gastroenterology* 1978;75:623-631.

576 SCHANG J.C., DEVROEDE G., DUGUAY C., HEMOND M., HEBERT M. Constipation par inertie colique et obstruction distale: étude électromyographique. *Gastroentérol. Clin. Biol.* 1985;9:480-485.

577 BINGHAM S., MCNEIL N.I., CUMMINGS J.H. The diet of individuals: a study of randomly-chosen cross section of British adults in a Cambridgeshire village. *Br. J. Nutr.* 1981;45:23-35.

578 BINGHAM S. Dietary fibre intake studies: problems, methods and results. In: TROWELL H.S., BURKITT D.P., HEATON K.W., eds. *Dietary fibre – fibre-depleted foods and disease*. 2d ed. London: Academic Press, 1985: 77-104.

579 BINGHAM S. Aspects of dietary fibre methodology. *Br. Nutr. Found. Bull.* 1985;10:90-103.

580 BINGHAM S. Dietary fibre consumption and its association with large bowel cancer in man. *Med. Oncol. Tumour Pharmacother.* 1985;2:149-155.

581 BINGHAM S. Definitions and intakes of dietary fibre. *Am. J. Clin. Nutr.* 1987;45:1226-1231.

582 BINGHAM S., PETT S., DAY K.C. NSP intake of a representative sample of British adults. *J. Hum. Nutr. Diet.* 1990;3:333-337.

583 EMMETT P.M., SYMES C.L., HEATON K.W. Dietary intake and sources of non-starch polysaccharide in English men and women. *Eur. J. Clin. Nutr.* 1993;47:20-30.

584 RASMUSSEN H.S., HOLTUG K., ANDERSEN J.R., KRAG E., MORTENSEN P.B. The influence of ispaghula husk and lactulose on the in vivo and the in vitro production capacity of short-chain fatty acids in humans. *Scand. J. Gastroenterol.* 1987;22:406-410.

585 EDWARDS C.A., BOWEN J., BRYDON W.G., EASTWOOD M.A. The effects of ispaghula on rat caecal fermentation and stool output. *Br. J. Nutr.* 1992;68:473-482.

586 EASTWOOD M.A., BRYDON W.G., ANDERSON D.M.W. The effects of dietary gum karaya (sterculia) in man. *Toxicol. Lett.* 1983;17:159-166.

587 RAYMOND W.R., NAGEL C.W. Microbial degradation of gum karaya. *Carbohydr. Res.* 1973;30:293-312.

588 EASTWOOD M.A., SMITH A.N. Hydrophilic colloids in colonic motility. In: BERTACCINI G., ed. *Handbook of Experimental Pharmacology*. Berlin: Springer-Verlag, 1982: 323-346. (vol 59/II).

589 OPCS. *Office of Population Censuses & Surveys,* 1993. (OPCS Monitor DH2 93/2).

590 IARC/WHO, eds. *Cancer Incidence in Five Continents.* Lyon, France: International Agency for Research on Cancer. 1992. (IARC Scientific Publications No. 120).

591 POTTER J.D. Reconciling the epidemiology, physiology and molecular biology of colon cancer. *J. Am. Med. Ass.* 1992;268:1573-1577.

592 FEARON E.R., VOGELSTEIN B. A genetic model for colorectal tumorigenesis. *Cell* 1990;61:759-767.

593 BINGHAM S.A. Diet and large bowel cancer. *J. R. Soc. Med.* 1990;83:420-422.

594 TROCK B., LANZA E., GREENWALD P. Dietary fiber, vegetables and colon cancer. Critical review and meta analysis of the epidemiologic evidence. *J. Natl. Cancer Inst.* 1990;82:650-661.

595 IARC, ed. Alcohol drinking. *IARC Monogr. Carcinog. Risks Hum.* 1988;44:1-416.

596 RIBOLI E., CORNEE J., MACQUART-MOULIN G., KAAKS R., CASAGRANDE C., GUYADER M. Cancer and polyps of the colorectum and lifetime consumption of beer and other alcoholic beverages. *Am. J. Epidemiol.* 1991;134:157-166.

597 BINGHAM S.A. Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. *Proc. Nutr. Soc.* 1990;49:153-171.

598 GERHARDSSON M., NORELL S.E., KIVIRANTA H., PEDERSEN N.L., AHLBOM A. Sedentary jobs and colon cancer. *Am. J. Epidemiol.* 1986;123:775-780.

599 GERHARDSSON M., FLODERUS B., NORELL S.E. Physical activity and colon cancer risk. *Int. J. Epidemiol.* 1988;17:743-746.

600 GERHARDSSON M., STEINECK G., HAGMAN U., RIEGER A., NORELL S.E. Physical activity and colon cancer: a case-referent study in Stockholm. *Int. J. Cancer* 1990;46:985-989.

601 HERRERA L., KAKATI S., GIBAS L., PIETRZAK E., SANDBERG A.A. Brief clinical report: Gardner syndrome in a man with an interstitial deletion of 5q. *Am. J. Med. Genet.* 1986;25:473-476.

602 BODMER W.F., BAILEY C.J., BODMER J. *et al.* Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987;328:614-616.

603 KINZLER K.W., NILBERT M.C., SU L.K. *et al.* Identification of FAP locus genes from chromosome 5q21. *Science* 1991;253:661-665.

604 NISHISHO I., NAKAAMURA Y., MIYOSHI Y. *et al.* Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 1991;253:665-669.

605 SOLOMON E., VOSS R., HALL V. *et al.* Chromosome 5 allele loss in human colorectal carcinomas. *Nature* 1987;328:616-618.

606 GRIFFITHS D.F.R., SACCO P., WILLIAMS D., WILLIAMS G.T., WILLIAMS E.D. The clonal origin of experimental large bowel tumours. *Br. J. Cancer* 1989;59:385-387.

607 FULLER C.E., DAVIES R.P., WILLIAMS G.T., WILLIAMS E.D. Crypt restricted heterogeneity of goblet cell mucus glycoprotein in histologically normal human colonic mucosa: a potential marker of somatic mutation. *Br. J. Cancer* 1990;61:382-384.

608 VOGELSTEIN B., FEARON E.R., HAMILTON S.R. *et al.* Genetic alterations during colorectal-tumor development. *New Engl. J. Med.* 1988;319:525-532.

609 AALTONEN L.A., PELTOMAKI P., LEACH F.S. *et al.* Clues to the pathogenesis of familial colorectal cancer. *Science* 1993;260(5109):812-816.

610 PELTOMAKI P., AALTONEN L.A., SISTONEN P. *et al.* Genetic mapping of a locus predisposing to human colorectal cancer. *Science* 1993;260(5109):810-812.

611 Hill M.J., CROWTHER J.S., DRASAR B.S., HAWKSWORTH G., ARIES V., WILLIAMS R.E.O. Bacteria and aetiology of cancer of the large bowel. *Lancet* 1971;1:95-100.

612 HILL M.J., DRASAR B.S., WILLIAMS R.E.O. *et al.* Faecal bile acids and clostridia in patients with cancer of the large bowel. *Lancet* 1975;1:535-539.

613 REDDY B.S., SIMI B., ENGLE A. Biochemical epidemiology of colon cancer: effect of types of dietary fiber on colonic diacylglycerols in women. *Gastroenterology* 1994;106:883-889.

614 NEWMARK H.L., WARGOVICH M.J., BRUCE W.R. Colon cancer and dietary fat, phosphate and calcium: a hypothesis. *J. Natl. Cancer Inst.* 1984;72:1323-1325.

615 WELBERG J.M.M., KLEIBEUKER J.H., VAN DER MEER R., MULDER N.H., DE VRIES E.G.E. Calcium and the prevention of colon cancer. *Scand. J. Gastroenterol.* 1991;26(Suppl. 188):52-59.

616 ARMSTRONG B., DOLL R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. *Int. J. Cancer* 1975;15:617-631.

617 FELTON J.S., KNIZE M.G., SHEN N.H. *et al.* The isolation and identification of a new mutagen from fried ground beef: 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhIP). *Carcinogenesis* 1986;7(7):1081-1086.

618 GROSS G.A. Simple methods for quantifying mutagenic heterocyclic aromatic amines in food products. *Carcinogenesis* 1990;11(9):1597-1603.

619 SUGIMURA T., NAGAO M., WAKABAYASHI K. Heterocyclic amines in cooked foods: candidates for causation of common cancers. *J. Natl. Cancer Inst.* 1994;86(Editorial):2-4.

620 WATANABE M., OHTA T. Analysis of mutational specificity induced by heterocyclic amines in the lacZ gene of Escherichia coli. *Carcinogenesis* 1993;14:1149-1153.

621 BINGHAM S.A., PIGNATELLI B., POLLOCK J.R.A. *et al.* Does increased endogenous formation of N-nitrosocompounds in the human colon explain the association between red meat and colon cancer? *Carcinogenesis* 1996;17:513-523.

622 KADLUBAR F.F., BUTLER M.A., KADERLIK K.R., CHOU H.-C., LANG N.P. Polymorphisms for aromatic amine metabolism in humans: relevance for human carcinogenesis. *Environ. Health Perspect.* 1992;98:69-74.

623 MACLEAN K.N., SEE C.G., MCKAY I.A., BUSTIN S.A. The human immediate early gene BRFI maps to chromosome 14Q22-Q24. *Genomics* 1995;30(1):89-90.

624 VAN MUNSTER I.P., NAGENGAST F.M., TANGERMAN A. The effect of resistant starch on fecal bile acids, cytotoxicity and colonic mucosal proliferation. *Gastroenterology* 1993;104:A460.