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Session: Short-chain fatty acids

Regulation of short-chain fatty acid production

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Short-chain fatty acid (SCFA) formation by intestinal bacteria is regulated by many different host, environmental, dietary and microbiological factors. In broad terms, however, substrate availability, bacterial species composition of the microbiota and intestinal transit time largely determine the amounts and types of SCFA that are produced in healthy individuals. The majority of SCFA in the gut are derived from bacterial breakdown of complex carbohydrates, especially in the proximal bowel, but digestion of proteins and peptides makes an increasing contribution to SCFA production as food residues pass through the bowel. Bacterial hydrogen metabolism also affects the way in which SCFA are made. This outcome can be seen through the effects of inorganic electron acceptors (nitrate, sulfate) on fermentation processes, where they facilitate the formation of more oxidised SCFA such as acetate, at the expense of more reduced fatty acids, such as butyrate. Chemostat studies using pure cultures of saccharolytic gut micro-organisms demonstrate that C availability and growth rate strongly affect the outcome of fermentation. For example, acetate and formate are the major bifidobacterial fermentation products formed during growth under C limitation, whereas acetate and lactate are produced when carbohydrate is in excess. Lactate is also used as an electron sink in *Clostridium perfringens* and, to a lesser extent, in *Bacteroides fragilis*. In the latter organism acetate and succinate are the major fermentation products when substrate is abundant, whereas succinate is decarboxylated to produce propionate when C and energy sources are limiting.

Short-chain fatty acids: Intestinal bacteria: Fermentation: Carbohydrate breakdown: Protein digestion: Inorganic terminal electron acceptors

Short-chain fatty acids (SCFA) are the major end products of bacterial metabolism in the human large intestine. They are formed principally from polysaccharide, oligosaccharide, protein, peptide and glycoprotein precursors by anaerobic micro-organisms (Cummings & Macfarlane, 1991), although in quantitative terms carbohydrates are the most important SCFA progenitors. A wide range of bacterial hydrolytic enzymes depolymerise these large macromolecules, allowing the organisms to ferment their component sugars (Salyers & Leedle, 1983; Salyers, 1984). Starches that escape digestion in the small bowel, and plant cell-wall polysaccharides (celluloses and non-cellulosic polysaccharides such as pectins, xylans, arabinogalactans, gums and mucilages) are the main fermentation substrates (Macfarlane & Cummings, 1991).

The principal SCFA that result from both carbohydrate and amino acid fermentation are acetate, propionate and butyrate, although formate, valerate, caproate and the branched-chain fatty acids isobutyrate, 2-methyl-butyrate and isovalerate, which are formed during the catabolism of branched-chain amino acids (valine, leucine, isoleucine), are also produced in lesser amounts (Macfarlane & Macfarlane, 1995). Other fermentation products such as lactate, ethanol and succinate, which are intermediates in the global fermentation process in the microbiota, are to varying extents metabolised to SCFA by cross-feeding species in the ecosystem, and they do not usually accumulate to a substantial extent in the bowel (Bernalier *et al*. 1999).

Abbreviation: SCFA, short-chain fatty acids.

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Host, environmental and microbiological factors that affect short-chain fatty acid production in the colon

As a result of depletion of C sources in the proximal large intestine, particularly readily-digestible carbohydrates, a progressive reduction in bacterial substrate availability occurs as food residues move towards the distal gut. This situation affects the types and amounts of SCFA produced, as does the length of time digestive material spends in the colon (Cummings, 1978; Cummings *et al.* 1979, 1992). *In vivo* and *in vitro* studies show that long transit times in the large intestine can have profound effects on bacterial physiology and metabolism, leading to protein breakdown and amino acid fermentation making an increased contribution to colon SCFA pools (Macfarlane *et al*. 1992, 1994, 1998).

SCFA concentrations are highest in the proximal large intestine, mainly because of greater carbohydrate availability. Studies using intestinal material obtained from human sudden death victims (Cummings *et al*. 1987) found that acetate:propionate:butyrate values were similar in different regions of the large intestine (about 57:22:21). However, as illustrated in Fig. 1, subsequent work demonstrated that these spot measurements of SCFA levels in bowel contents were really showing the balance between bacterial SCFA formation and the absorption of these metabolites in the colon, and that bacterial fermentation product formation varied quantitatively and qualitatively in different regions of the colon, particularly in relation to butyrate and products of dissimilatory amino acid metabolism (Macfarlane *et al*. 1994).

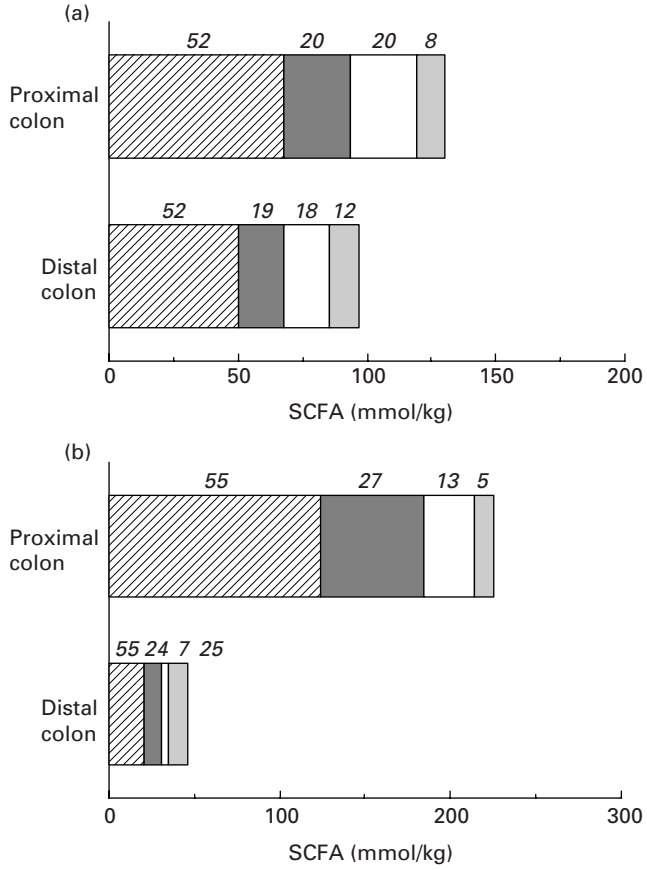

Fig. 1. (a) Short-chain fatty acid (SCFA) concentrations and relative amounts (mol; values shown in italics) in different regions of the large intestine and (b) rates of production and molar amounts (mol; values shown in italics) of SCFA produced from intestinal contents incubated for 48 h with no exogenous carbon sources, under anaerobic conditions. (▨), Acetate; (▤), propionate; (▭), butyrate; (⋈), branched-chain fatty acids.

In addition to the importance of gut transit time, mentioned earlier, many host-related factors affect bacterial metabolism and SCFA formation in the gut, including diet and other less direct determinants such as ageing, neuroendocrine system activity, stress, pancreatic and other secretions in the digestive tract, mucus production, disease, drugs, antibiotics and epithelial cell turnover times. From a microbiological viewpoint, the chemical composition, physical form and amount of substrate available affects bacterial fermentation reactions, which are also dependent on the types and numbers of different bacterial populations in the gut, catabolite regulatory mechanisms, the availability of inorganic electron donors, such as nitrate (Allison & Macfarlane, 1988) and sulfate (Gibson *et al*. 1993), as well as competitive and cooperative interactions between different species in the microbiota (Macfarlane & Gibson, 1994).

Results from fermentation studies

In vitro fermentation experiments with faecal bacteria have demonstrated that individual polysaccharides are broken down at different rates, e.g. starch and pectin are degraded more rapidly than xylan or arabinogalactan (Englyst *et al*. 1987). This finding is of relevance to bacterial catabolic processes in the gut because, to a large extent, substrate concentration regulates the way in which the organisms compete for fermentable substrate, as well as the control mechanisms involved in fermentation reactions (Degnan, 1992). These studies also demonstrated that fermentation of different polysaccharides gives rise to distinct patterns of SCFA production. For example, acetate was the main product of pectin and xylan breakdown, while large amounts of acetate and propionate were produced from arabinogalactan. Of the four substrates tested, butyrate was only formed in substantial amounts from starch.

Lactate was not produced in substantial amounts from pectin, xylan or arabinogalactan; however, *in vitro* and *in vivo* studies have shown that starch is an important precursor of this fatty acid (Macfarlane & Englyst, 1986; Etterlin *et al*. 1992). The majority of intestinal lactate-producing bacteria form the L-enantiomer, and it has been shown that the majority of this metabolite in the large gut is present in the proximal large bowel. That this situation is mainly a reflection of substrate availability in this region of the intestine was demonstrated by measurements of lactate in large bowel contents, which showed a positive correlation with residual starch concentrations in the caecum (Macfarlane & Gibson, 1994).

Fermentation strategies in saccharolytic gut micro-organisms

Although many different types of C source are used as fermentation substrates by bacteria growing in the large

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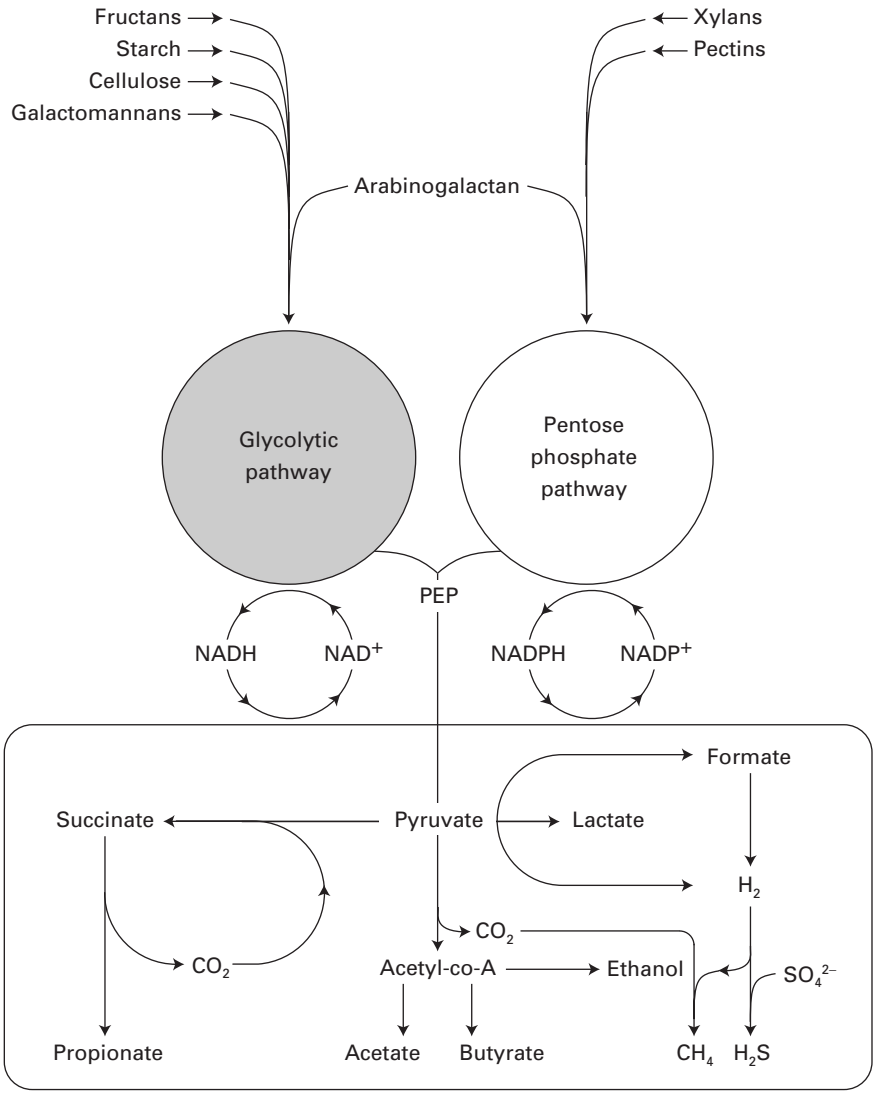

Fig. 2. Simplified diagram of polysaccharide breakdown and the main routes of carbohydrate fermentation in the large intestine. PEP, phospho*enol*pyruvate

intestine, these substances are catabolised in a relatively small number of biochemical pathways, as shown in the simplified scheme of carbohydrate metabolism in Fig. 2. The Entner–Doudoroff pathway is not used by human gut micro-organisms to ferment carbohydrates, while the fructose-6-phosphate shunt employed by bifidobacteria has a restricted taxonomic distribution in the colonic microbiota. The majority of intestinal bacteria use the glycolytic pathway to derive energy from carbohydrates, which are initially converted to pyruvate and acetyl-CoA. These metabolites are key control points in fermentative metabolism, which can be converted into a wide range of products (Macfarlane & Gibson, 1996).

The pentose phosphate pathway also occurs in many gut bacteria, where it is used in the dissimilatory metabolism of pentoses (see Fig. 2) or to produce NADPH and pentoses for biosynthetic purposes. During the breakdown of hexoses, glucose-6-phosphate is oxidatively decarboxylated to ribulose-5-phosphate, which subsequently undergoes a series of non-oxidative interconversions catalysed by transketolases and transaldolase, in which triose and hexose phosphates are formed. In dissimilatory metabolism these intermediates feed into various levels of the glycolytic pathway, leading ultimately to the synthesis of pyruvate.

Anaerobic chemoheterotrophs in the colon can be placed into two broad groups: species that are restricted to fermentative metabolism and ATP formation by substrate-level phosphorylation; bacteria that are able to carry out anaerobic respiration. Substrate-level phosphorylation reactions do not employ respiratory chains that use terminal electron acceptors such as oxygen or inorganic ions (e.g. nitrate, sulfate); this function is usually undertaken by organic compounds derived from the original fermentation substrate. Consequently, fermentation reactions must be self-balancing with respect to the formation and consumption of reducing power, with the redox difference between substrates and end products determining the amount of energy that can be produced. Thus, fermentation reactions that result in large amounts of acetate being formed generally yield higher levels of ATP (Macfarlane, 1991).

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Substrate-level phosphorylation reactions are energetically inefficient when compared with oxidative metabolism, and they result in relatively low ATP yields. Large amounts of substrate are therefore required for growth in fermentative micro-organisms, leading to substantial levels of metabolic end products being formed (Macfarlane & Gibson, 1995).

Control of fermentation reactions in colonic bacteria

Fermentations are regulated by the need to maintain redox balance, which is mediated through the reduction and oxidation of pyridine nucleotides, ferredoxins and flavins. To a large extent, this process affects the flow of C through the bacteria, the energy yield that can be obtained from the substrate, and the fermentation products that are ultimately generated. Synthesis of reduced products including hydrogen, lactate, succinate, butyrate and ethanol is used to effect redox balance during fermentation (see Fig. 2), whereas the formation of more oxidised products, such as acetate, is associated with ATP production. Conversely, when intestinal micro-organisms form more reduced fermentation products, this process results in comparatively low ATP yields. As will be discussed later (p. 71), many gut anaerobes take advantage of the flexibility offered by branched fermentation pathways, which allow them to adapt the thermodynamic efficiency of substrate catabolism, in response to changing environmental conditions, through modulating ATP formation and redox balance.

Relatively few intestinal bacteria make succinate, but many species form ethanol as a major end product of metabolism. It is a highly reduced metabolite, which consumes two molecules of NADH per molecule of alcohol that is produced. Unlike the ethanol fermentation in yeasts, which oxidises only one molecule of NADH, and where the alcohol is produced from pyruvate by pyruvate decarboxylase and alcohol dehydrogenase, the vast majority of bacteria form ethanol from acetyl-CoA through the activities of acetaldehyde dehydrogenase and alcohol dehydrogenase. In ethanol-producing species that ferment carbohydrates by the glycolytic pathway, acetate must also be formed to maintain redox balance.

Effects of growth rate and nutrient availability on fermentation product formation

Chemostat studies in which pure cultures of colonic micro-organisms are grown under different environmental conditions can tell us much regarding the way intestinal bacteria control fermentation product formation. This information can be seen in Table 1, where three species of gut bacteria are used as examples to show how individual micro-organisms approach the problem of substrate utilization and energy generation. For example, when *Clostridium perfringens* was grown under C-excess conditions, the bacteria increased lactate formation at the expense of acetate, especially at high specific growth rates. These experiments demonstrated that the organism optimised energy gain during growth under carbohydrate-limiting conditions by producing more ATP from acetyl phosphate, whereas when substrate was in excess, the reduction in acetate formation lowered the efficiency of energy transduction, while providing for a high rate of C flow through the cells, by using lactate as an electron sink.

In *Bifidobacterium breve* growth rate had little effect on metabolic end products, although carbohydrate availability markedly influenced the outcome of fermentation. Thus, formate and acetate were the main end products of metabolism during C-limited growth, while under conditions of carbohydrate excess, acetate and lactate were formed. In a manner analogous to the situation in *C. perfringens*, this observation can be explained by the fact that lactate was used as an electron sink to dispose of excess reducing power when the supply of substrate was plentiful, whereas metabolism was directed towards energy production under C-limited growth conditions. This system operates because

Table 1. Effect of growth rate and carbohydrate availability on fermentation product formation in continuous cultures of intestinal bacteria (from Macfarlane & Gibson, 1995)

		Relative amounts of fermentation products (mol)						
Bacterium	Hourly specific growth rate (μ)	Formate	Acetate	Propionate	Butyrate	Succinate	Ethanol	Lactate
Clostridium perfringens								
C-limited	0·04	–	74	–	17	6	–	3
	0·16	–	80	–	8	ND	–	12
C-excess	0·04	–	49	–	12	5	–	35
	0·16	–	18	–	3	ND	–	80
Bifidobacterium breve								
C-limited	0·10	32	59	–	–	–	8	ND
	0·45	29	60	–	–	–	10	ND
C-excess	0·09	ND	70	–	–	–	3	25
	0·60	ND	68	–	–	–	6	25
Bacteroides ovatus								
C-limited	0·06	–	58	33	–	9	–	ND
	0·19	–	53	8	–	39	–	ND
C-excess	0·06	–	49	15	–	31	–	5
	0·19	–	49	11	–	36	–	4

ND, Not detected.

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pyruvate can be metabolised by two routes: it can be reduced by NADH to produce lactate; alternatively, pyruvate can undergo phosphoroclastic cleavage to give formate and acetyl phosphate. Half the resulting acetyl phosphate must be reduced to ethanol to facilitate oxidation of NADH produced earlier in the metabolic pathway, while the remainder is available to make extra ATP through the formation of acetate (DeVries & Stouthamer, 1968).

In the case of *Bacteroides ovatus* both growth rate and C availability affected fermentation when metabolism was C limited. Acetate formation was maximal under energy limitation to maximise ATP yields and, by producing succinate, *B. ovatus* disposed of reducing equivalents generated during glycolysis while, in addition, the oxidation of fumarate to succinate allowed the bacterium to synthesise extra ATP. The organism formed propionate by decarboxylation of succinate, which serves to regenerate CO_2 for use in converting C_3 acids to C_4 acids in the succinate pathway. When C was in excess, acetate and succinate were the principal end products of metabolism, and this outcome was unaffected by growth rate, whilst acetate and propionate predominated in C-limited chemostats. These reactions are controlled by intracellular levels of CO_2, which is produced during the conversion of pyruvate to acetate, and is therefore dependent on carbohydrate availability and the activity of the glycolytic pathway. When sufficient carbohydrate is present, there is a reduced need to decarboxylate succinate and this metabolite accumulates instead of propionate (Macy *et al*. 1978).

Effect of inorganic anions on fermentation

Nitrate and sulfate have been shown to profoundly affect fermentation reactions during carbohydrate breakdown under anaerobic conditions. This effect is typically manifested in the form of increased acetate production, together with reduced butyrate and lactate formation (Allison & Macfarlane, 1988; Gibson *et al*. 1993). This process occurs because these inorganic anions act as electron sinks, which enables some intestinal bacteria to use hydrogen as an electron donor in metabolism. Fig. 3 shows a highly-simplified scheme of how acetate, butyrate, lactate and hydrogen are produced in saccharolytic clostridia. Hexoses are catabolised in the glycolytic pathway to pyruvate and acetyl-CoA. Lactate formation from pyruvate can be used to regenerate oxidised pyridine nucleotides, which enables glycolysis to continue or, alternatively, hydrogen can be formed from pyruvate by the enzyme pyruvate:ferredoxin oxidoreductase linked to hydrogenase (not shown). This reaction is exergonic and is generally unaffected by the partial pressure of H_2. However, the main route of H_2 formation occurs through the action of NADH:ferredoxin oxidoreductase and hydrogenase. This reaction is endergonic and consumes reduced pyridine nucleotides and requires a low partial pressure of H_2 to be thermodynamically feasible. This condition is made possible by the activities of syntrophic nitrate- and sulfate-reducing populations, as well as CH_4-producing and acetogenic bacteria (not shown). As NADH is consumed during H_2 formation, it is not available to convert acetoacetyl-CoA to butyryl-CoA,

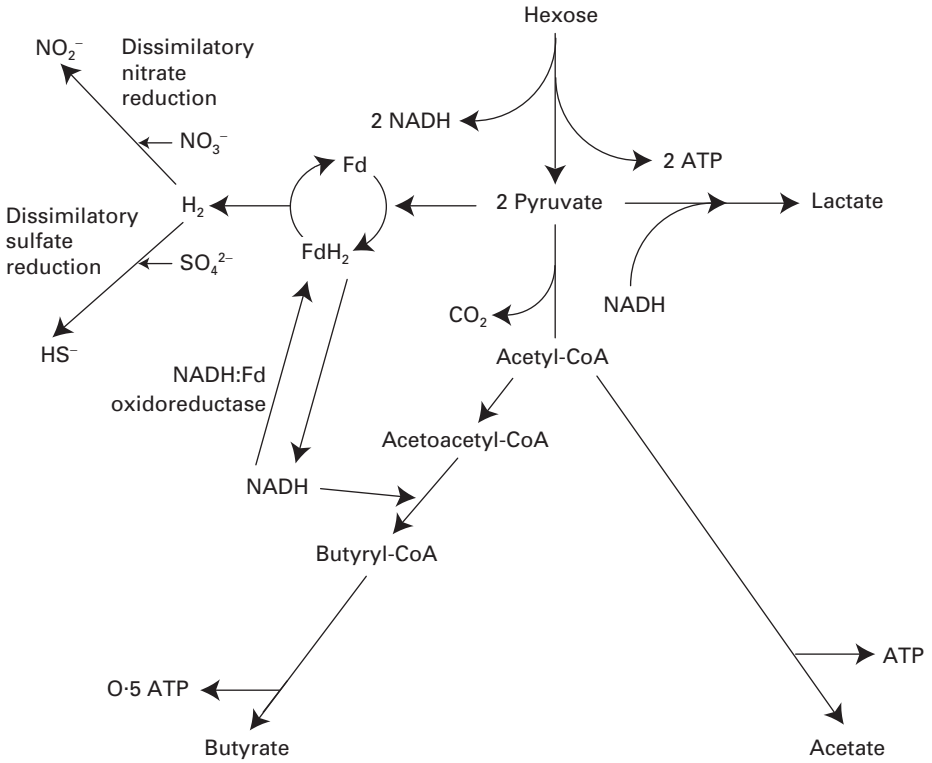

Fig. 3. Diagram of acetate, butyrate, lactate and hydrogen formation in saccharolyic clostridia, showing how syntrophic hydrogen-consuming populations in the gut interact with hydrogen metabolism and affect fermentation product formation. Fd, ferredoxin.

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and butyrate (and lactate) production is reduced. Acetyl-CoA is consequently routed into acetate formation, which is advantageous for the organism because ATP yields are twice as high as when butyrate is used as an electron sink.

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