Progress report

Short chain fatty acids in the human colon

The human colon contains a luxuriant mixed culture of bacteria, probably at least 175 g. The special property of these bacteria is their metabolism, which is in the main strictly anaerobic, the end-products being primarily the short chain, or volatile, fatty acids, acetic, propionic, and butyric acid. These are the principal known anions in the colon but, although this has often been reported,¹⁻⁴ little thought has been given to the possible implications.

Physiology

OCCURRENCE OF SHORT CHAIN FATTY ACIDS IN COLON

Acetate, propionate, and butyrate occur in the large intestine of all mammalian species so far studied. They are the predominant anions in herbivorous animals like the horse,⁵ kangaroo, rabbit, and guinea-pig,^{6,7} omnivores such as the pig,⁸ and even the dog, a carnivorous species.⁹ They are the major anions in human faeces¹ but no values have yet been reported of their concentrations in the rest of the colon. In addition to the large intestine short chain fatty acids are also found in the rumen where the complex metabolic pathways leading to their formation have been extensively studied (see review by Prins, 1977).¹⁰

Fermentation in the rumen is very similar to that in the colon and, given appropriate substrates, experimental evidence indicates that this pattern of metabolism takes place in the human colon.¹¹⁻¹³ The microflora of human faeces is similar to that of the rumen¹³ and reflects that of the rest of the colon.¹¹ Human colonic microflora are capable of fermentation reactions necessary for the production of short chain fatty acids.¹³⁻¹⁶ There is a close similarity in the molar ratios of short chain fatty acids in the colon amongst various species (Table). Concentrations of these acids in human faeces are, however, lower than those seen in the colon of the rat or pig and in the rumen. This may be a true species difference but is more likely to reflect differences between faecal and colonic contents.

Production and absorption rates are the primary factors controlling short chain fatty acid concentrations and molar ratios, although the rate of passage of digesta through the gut (transit) is also important.¹⁷⁻²³ The effect of diet is less significant than one would expect. For example, changing a dog from a cereal- to meat-based diet had virtually no effect on faecal and colonic short chain fatty acid levels,⁹ although in pigs a cereal-based diet led to higher acetate and lower propionate levels than a formula-type diet.²² Fasting reduces production and concentrations.¹⁷⁻²¹ In man,⁴ a change from an *ad libitum* diet to one containing only carbohydrate led to an overall fall in total short chain fatty acids concentration in faecal dialysate from 85 to 46 mmol/l and a small change in the molar ratios of acetate : propionate : butyrate from 59 : 22 : 19 to 68 : 17 : 13. When the

Table Short chain fatty acid concentrations in gut

<i>Species</i>	<i>Site</i>	<i>Acetate</i>	<i>Propionate</i>	<i>Butyrate</i>	<i>References</i>
		(mmol/kg)			
Rat	Caecum	101	57	32	Remsey and Demigne (1976) ¹⁶
	Colon	70	29	7	
	Faeces	75	27	16	
Pig	Caecum	118	68	25	Argenzio and Southworth (1974) ⁸
Sheep	Rumen	65	21	18	Hungate (1966) ¹⁸
Cow	Rumen	66	23	20	Hungate (1966) ¹⁸
Man	Faecal dialysate	41	12	13	Cummings <i>et al.</i> (1979) ¹⁹
		46	17	15	Rubinstein <i>et al.</i> (1969) ⁴
		39	19	14	Bjork <i>et al.</i> (1976) ²⁰
	Faecal water	93	46	24	Zilstra <i>et al.</i> (1977) ²¹
	Faeces	48	11	5	
<i>Molar ratios (%)</i>					
Pig	Faeces	66	22	7	Sambrook (1979) ²²
	Caecum	56	32	12	Argenzio and Southworth (1974) ⁸
Rat	Caecum	61	25	14	Remsey and Demigne (1976) ¹⁷
Sheep and cow	Rumen	62	21	16	Hungate (1966) ¹⁸
Man	Faeces	60	24	16	*

*Average value from Rubenstein *et al.* (1969)⁴; Bjork *et al.* (1976)²⁰; Zijlstra *et al.* (1977)²¹; Cummings *et al.* (1979)¹⁹.

same subjects took only methyl cellulose an additional fall in concentration was seen (to 23 mmol/l) and the molar ratios were 60 : 24 : 15. Calorie intake was considerably reduced, however, in the experimental diets. In a different study¹⁹ no significant change in short chain fatty acid concentrations was seen when four healthy subjects went from a low protein (66±4.6 (SEM) mmol/l) to an equicaloric high protein diet (58±4.7 mmol/l) or when 39 g of dietary fibre were added to the high protein diet (66±4.2 mmol/l).

SOURCES AND METABOLISM OF SHORT CHAIN FATTY ACIDS

Short chain fatty acids are produced by fermentation from carbohydrate. The major source of this in the human colon is thought to be plant cell-wall polysaccharides such as cellulose, pectins, and hemicelluloses, currently referred to in human nutrition as dietary fibre. Starch would also be a suitable substrate if it were to reach the colon in significant quantities. Plant cell-wall polysaccharides are composed of hexose (glucose and galactose), pentose (xylose and arabinose), and uronic acid monomers which are fermented by gut micro-organisms along a variety of pathways. The important feature to remember of this metabolism is that it is anaerobic.

Hexose breakdown is mainly *via* the Embden-Myerhoff-Parnas glycolytic pathway to pyruvate. Alternatively, hexose is converted to 6-phospho-

gluconate and then metabolised mainly by the pentose phosphate cycle.¹⁰ This latter route, a major one in some aerobic bacteria, is also used for the conversion of pentoses to glycolytic intermediates and intermediates essential for the synthesis of nucleic acids, cofactors, and amino acids. Pyruvate is the main metabolite of these fermentation reactions but very little is found in the gut because it is converted to a series of end products—namely, acetate, propionate, butyrate, carbon dioxide, hydrogen, methane, and water. Acetate is usually formed by the oxidative decarboxylation of pyruvate and butyrate by reduction of acetoacetate formed from acetate. The production of propionate is by two main, but circuitous, routes, firstly, involving fixation of CO₂ to form succinate which is subsequently decarboxylated (the 'dicarboxylic acid pathway') or, secondly, from lactate and acrylate (the 'acrylate pathway').¹⁰

Lactate (both D and L) may be formed from pyruvate by many gut anaerobes but is not a key intermediate in fermentation and significant amounts are rarely found in the human or animal gut, except in the pig's stomach.⁶ When, however, flux through the glycolytic pathway is high, as when large quantities of readily fermentable soluble carbohydrate are available, lactate production is favoured. Increased fermentation rates also result in a lowering of pH which in turn inhibits the metabolism of organisms which utilise lactate. Thus lactate levels may become significant.^{25–26} Up to 50 mmol/l lactate levels in faeces have been reported in infants suffering from acute infectious diarrhoea when fed on milk²⁷ and in children with deficiency of sugar-splitting enzymes in the small gut.^{28–29} In these circumstances malabsorption of sugar favours colonic lactate production, although acetate usually remains the principal anion. In man D-lactic-acidosis has been reported in a patient with the short bowel syndrome³⁰ where circumstances would dictate similar fermentation patterns in the colon.

Other intermediates in the anaerobic breakdown of complex carbohydrates in the rumen and colon are hydrogen, ethanol, and formate. Formate is not found in significant amounts because it is rapidly converted to hydrogen and carbon dioxide.¹⁵ In human faeces formate concentrations are very low (1–2 mmol/l).⁴ Ethanol (and methanol) are produced in relatively small quantities and are absorbed without additional microbial metabolism. High levels (90–130 mmol/l) have been reported in the caecum of the ptarmigan³¹ and, as with lactate, alcohol production is said to be favoured by acid pH.¹⁵

Hydrogen is a key intermediate in any fermentation system but concentrations in the rumen are low because it is rapidly converted to methane by direct reduction of CO₂ by methanogenic bacteria. Methane may also be produced from a number of other substrates including acetate but this is seen only when retention of material in the system is four to five days or longer.³² Both hydrogen and methane are produced in man.^{33–36} In clear distinction from the rumen, however, only about 30–60% of humans produce methane, at least of those examined in the USA^{34–37} and UK.³⁸ These differences may possibly be due to the smaller amounts of CO₂ available for fermentation in the 'Westernised' colon or to differences in transit time. About 75–80% of Nigerians produce methane (Tompkins, Drasar, Wiggins, Bradley, and Gyselynek; personal communication) which

may be due to their much higher intake of complex carbohydrate and thus more typically rumen-like fermentation in the caecum and colon.

TOTAL DAILY PRODUCTION OF SHORT CHAIN FATTY ACIDS

There can be little doubt therefore that a fermentative system similar to that of the rumen occurs in the colon of many animals including man. Its importance, in terms of overall metabolism, will depend on the amount and type of substrate degraded and on the fate of the short chain fatty acids. Fermentation has been quantified in the rumen and an overall equation for the anaerobic breakdown of complex carbohydrate derived:¹⁵

Miller and Wolin (1979)¹² have derived a similar equation for fermentation in the human colon based on the molar ratios of short chain fatty acids in faeces and known production of carbon dioxide and methane:

This equation assumes that short chain fatty acids are the sole non-gaseous end-product, but takes no account of non-methane producers.

How can colonic fermentation be quantified in man? Two possible approaches can be made, both assuming the rumen data to be approximately correct for the colon. Firstly, if the amount of substrate available for fermentation is known then short chain fatty acid production can be calculated. The amount of dietary carbohydrate, presumably the main substrate for the bacteria, which is fermented in the colon each day is around 20 g. About 15 g from dietary fibre³⁰ and 5–6 g of soluble carbohydrate.^{10–11} From equation (2) this would yield about 200 mmol short chain fatty acids. This figure will be an underestimate in people consuming more dietary fibre than the UK population^{12–13} or if other carbohydrate substrates are available.

An alternative approach, used by Smith and Bryant,³² is based on the amount of bacteria excreted in human faeces each day. *In vitro* fermentation studies have demonstrated the amount of carbohydrate needed to provide energy for the growth of anaerobic bacteria. One mole of hexose, metabolised anaerobically, yields 5 mol ATP.^{23–24} The theoretical yield of bacterial cells (dry) is 26 g/mol ATP.^{44–45} This falls to about 20 g/mol when the cost of maintenance energy for bacteria is taken into account at a turnover time of about 48 hours.²³ On average for each mole of hexose fermented, about 62 g of bacterial cells are produced, or 34% of the weight of carbohydrate.¹⁶ Smith and Bryant³² suggest, on theoretical grounds, that for man, bacteria produced are equivalent to 'at least 13% of the weight of carbohydrate utilised anaerobically', which is a much lower figure. In fact, the actual yield of bacterial cells per gram of hexose fermented varies widely depending on the availability of pre-formed monomers (such as amino acids) for biosynthesis, cell wall composition, and maintenance energy requirements of the bacteria. Much is known about this in the rumen^{11–12} but little for man, which makes it

difficult to predict accurately the substrate requirements for bacterial growth in the colon.

However, the amount of bacterial cells produced in the colon in man is known, and can be used as a basis for calculating short chain fatty acid yields. Stephen and Cummings^{17, 18} have shown that bacterial cell yields (dry weight) from fermentation of dietary fibre from cabbage are 28% of the weight of fibre fermented and for wheat bran 36%.¹⁸ In these studies it was assumed that the only carbohydrate available in the colon, as a result of the experimental dietary changes, was non-starch polysaccharide from the cell wall material. Were other carbohydrate to be carried into the colon as a result of the dietary changes, then these apparent yields would be overestimates. Nevertheless, they are remarkably close to rumen values. Daily excretion of bacterial cells in man is about 15 g while on an average United Kingdom diet, although it can be considerably more than this.¹⁹ If a conversion factor of 25% is taken, this is equivalent to 60 g carbohydrate fermented daily and a yield of 600 mmol short chain fatty acids. This is an intriguing approach. It is not currently thought that so much carbohydrate is metabolised in the colon. However, anaerobically, bacteria get much less energy for growth from protein and one would have to assume the whole of normal protein intake to pass to the colon to sustain microbial growth, an unlikely occurrence. Long chain fatty acids are not used by anaerobes as an energy source.¹⁰ To sustain known bacterial growth either more carbohydrate passes into the human colon each day than is currently believed, or the yield of bacterial cells per g of carbohydrate is higher than that in the rumen. Alternative possible sources of carbohydrate other than dietary fibre available for breakdown to provide energy for bacterial growth include unabsorbed starch, which recent studies reported by Anderson *et al.*²⁰ suggest could be an important contributor, or mucous secretion.

Whatever the possible source, if the energy requirements for the growth of 15 g of bacteria in the gut are met each day by anaerobic fermentation then a considerable amount of short chain fatty acids are produced. What is their fate? These acids are the end products of carbohydrate fermentation. With the exception of small amounts of acetate being converted to methane in subjects who have slow transit times, they must either be excreted in the faeces or absorbed. In the adult human with a faecal output of 80–230 g/day only 7–20 mmol/day are excreted²¹ so most short chain fatty acids must be absorbed.

ABSORPTION

There can be little doubt that short chain fatty acids are absorbed from the human colon. Were they not, man would be unique in the animal kingdom. All mammalian species studied absorb these acids including the rat,^{17, 22} pig,^{8, 23} horse,²⁴ and goat.²⁴ Absorption of all three short chain fatty acids from the human colon has been shown.^{25–27} McNeil²⁸ measured absorption of these acids in the human rectum and descending and transverse colon and showed that absorption rates are comparable with those observed in animals—for example, human 6.1–12.6 $\mu\text{mol}/\text{cm}^2/\text{h}$; horse 8 $\mu\text{mol}/\text{cm}^2/\text{h}$;²⁹ pig 8–10 $\mu\text{mol}/\text{cm}^2/\text{h}$,⁸ and from the rumen 10.5 $\mu\text{mol}/$

cm²/h.⁶⁰⁻⁶¹ Net movement out of the colonic lumen of short chain fatty acids is more rapid than net sodium transport.⁵⁴⁻⁵⁶

The exact mechanism whereby these acids are absorbed still requires additional study in all species, but a number of general characteristics emerge from published reports. Transport from the lumen is invariably associated with the appearance of HCO₃⁻, with the stimulation of sodium absorption (except in the horse)⁵⁹ and is independent of bulk water flow.⁵³⁻⁵⁴ Important unanswered questions in man include whether they are absorbed primarily as acids or in the ionised form, whether there is a specific membrane carrier for short chain fatty acids, how the transport of these acids affects that of other ions and, more particularly, the influence of mucosal short chain fatty acid metabolism on their transport.

Role of bicarbonate

An understanding of HCO₃⁻ metabolism must hold the key to the mechanism of short chain fatty acid transport, as it appears consistently in the lumen during absorption of these acids. HCO₃⁻ accumulation is independent of the chloride/HCO₃⁻ exchange known to occur in the human ileum and colon,⁶²⁻⁶³ as its appearance continues when chloride is replaced in the lumen by sulphate.⁶⁴ In both the rat⁵²⁻⁶³ and pig colon⁵³ HCO₃⁻ appearance is independent of chloride/HCO₃⁻ exchange. On a molar basis HCO₃⁻ appearance is equal to about half total short chain fatty acid absorption in the rumen⁶⁶⁻⁶⁷ and colon.⁵³⁻⁵⁶⁻⁵⁷

The source of HCO₃⁻ is either plasma, or hydration of CO₂ with carbonic anhydrase (CA):

In those parts of the gut where fermentation occurs—that is, rumen, caecum, and colon—the epithelia contain high concentrations of carbonic anhydrase,⁶⁸⁻⁷¹ although levels in the human colon have yet to be reported. *Intracellular* HCO₃⁻ could be exchanged for the ionised form of short chain fatty acids across the luminal border of the cell in a way analogous with chloride exchange. With an average pKa of 4.8 short chain fatty acids will be about 95% ionised at the pH of human caecum (6.0) and left colon (7.0).⁷² The rise in luminal pH seen when these acids are absorbed from all parts of the human gut⁵⁶⁻⁵⁷⁻⁶⁴⁻⁷³⁻⁷⁴ and in the horse⁵⁹ and pig⁵³ would fit with HCO₃⁻ exchange because the accumulation in the lumen of HCO₃⁻ would shift the reactions shown in equation (3) to the left. This would result in a rise of pCO₂ which has been observed in the rat⁵² and horse⁵⁹ but in the pig,⁵³ the rumen,⁶⁶ and man⁵⁷ pCO₂ falls.

In fact, gut mucosa is relatively impermeable to the ionised form of these acids,⁷⁵ so an alternative and more likely mechanism of absorption of short chain fatty acids has been suggested.⁵³⁻⁵⁷⁻⁵⁹⁻⁷⁴ In this mechanism *luminal* hydration of CO₂ occurs and allows protonation of the acid and its rapid absorption by diffusion in the unionised form. This would lead to HCO₃⁻ accumulation and a fall in pCO₂ as observed in man, and in the pig whose colon is similar to the human. Absorption in the unionised form would be additionally favoured by the acid microclimate at the colonic mucosal surface,⁷⁶⁻⁷⁷ although this is currently disputed.⁷⁸

Sodium absorption

Another clue to the mechanism of short chain fatty acid absorption lies in the stimulation of sodium absorption seen during acetate transport in several species.^{52-55 57 70} In the rat colon it is notable that neither succinate nor lactate, which are poorly absorbed, stimulate sodium absorption, whereas acetate does. The most likely interaction between short chain fatty acids and sodium would be through a recycling of hydrogen ion (H^+). Transport of the unionised acid into the cell drives Na^+-H^+ exchange and stimulates sodium absorption. In the studies of Argenzio and Whipp⁵³ of the perfused pig colon marked differences in luminal pCO_2 and pH were observed when either Na_2SO_4 or CH_3COONa was perfused. Perfusion with Na_2SO_4 was associated with a fall in HCO_3^- concentration, rise in pCO_2 , and acidification of the luminal solution; this suggests that net Na absorption is associated with H^+ secretion. With sodium acetate the reverse is seen. The secreted H^+ ion facilitates absorption of the unionised acid. However, in man an Na-H exchange is not thought to occur in the colon⁶³ and perfusion of Na_2SO_4 solutions through the isolated human colon does not lead to luminal acidification.⁶⁰

Several other possibilities for the control of short chain fatty acid absorption have been postulated. One proposed by Jackson⁶¹ depends on the development of an area of high pH within the mucosa, probably in the intracellular space. This would facilitate the absorption of weak acid. On the other hand Lamers⁶² has shown evidence for the existence of a carrier for monocarboxylate ions across the gut mucosa and suggested that this was likely to be situated in the basolateral border of the epithelial cell.

These disparate observations indicate that a great deal more needs to be learnt about the transport of short chain fatty acids across the human colonic mucosa. Both species differences and those in transport at various levels in the gut make the development of a unifying hypothesis difficult. It is remarkable, however, that the study of electrolyte transport in the human colon should have proceeded for more than 20 years, yet only recently has attention been given to the role of short chain fatty acids, the major anion in the colon, and one which clearly affects both sodium and bicarbonate transport.

MUCOSAL METABOLISM

Study of the transport of short chain fatty acids across colonic mucosa is complicated by the fact that substantial amounts are metabolised within the epithelial cell itself. In the rumen absorption rates of short chain fatty acids increase with chain length (acetate < propionate < butyrate), but the amount transported to the blood decreases,⁶⁰ differences attributable to cellular metabolism. Ketone body production (acetoacetate and β -hydroxy butyrate) accounts for 30% of acetate metabolism, 75% of butyrate, and some conversion of butyrate to acetate occurs.⁶¹ In the sheep Bergman²¹ has shown that 50% of absorbed propionate and 90% of butyrate are metabolised in the mucosa, the rest being almost entirely cleared by the liver. Very little propionate and butyrate are found in the arterial blood of ruminants. In addition 24-35% of acetate is removed from the blood during passage through portal viscera (18% of acetate turnover).

Much less information is available about short chain fatty acid metabolism by caecal and colonic mucosa. In the pig substantial amounts (over 50%) of total short chain fatty acids appear to be used by the mucosa¹⁶ but in the caecum of rabbits⁷ and rats¹⁷ only about 12% of butyrate is converted to ketone bodies and virtually no conversion occurs in the mucosa of the colon. In man, using isolated human colonic epithelial cells, Roediger¹² has shown that butyrate is an important energy source for colonic mucosa, accounting for the major part of energy needs even in the presence of glucose.

Measurement of short chain fatty acid absorption from colon

As adequate methods are available for chemical estimation of these acids¹³ most of the problems in studying their metabolism in the colon arise from difficulties in studying this organ. A variety of techniques have been applied to measuring short chain fatty acid movement in the colon, including *in vitro* tissue studies, colonic perfusion, and the dialysis method.

Colonic mucosal electrolyte transport has been measured *in vitro* using modifications of the Ussing technique.^{63-64, 85} This method has the advantage of allowing bidirectional fluxes of ions across the mucosa to be measured directly and to isolate the effect on electrolyte transport on the high spontaneous electrical potential difference found *in vivo*. Problems in interpretation arise from difficulties with the viability of tissue and the fact that it is removed from its normal neural and hormonal controls. It is useful, however, for the study of mechanisms of electrolyte transport and, while no data have yet been published of its use for human short chain fatty acid absorption, the techniques have been applied to animal studies.⁵⁹ An alternative *in vitro* method using isolated colonic epithelial cells has recently been reported by Roediger and Truelove.⁶⁶ The metabolism of individual colonocytes can be studied and already the technique has yielded valuable information on short chain fatty acid metabolism by these cells.⁶²

In vivo perfusion of the whole colon has been established over a number of years^{7, 58} and has been applied to the study of short chain fatty acid absorption.⁵⁷ The advantages of being able to study colonic function directly *in vivo* are obvious and the technique has provided useful information on overall colonic absorptive capacity. There are, however, problems with contamination of perfusing solution by ileal effluent, of reflux of perfusate into the ileum, in validating steady state conditions, in the large amounts of isotope needed for unidirectional flux studies and, particularly for short chain fatty acid metabolism, of contamination of solutions with retained faecal material. The technique has not been successfully applied to individual segments of the colon, and, as colonic function varies from region to region,^{58, 60} this information is lost. Some of these problems can be overcome by perfusion of the isolated whole colon, either defunctioned^{1, 55} or surgically isolated,^{6, 64} but the precision of the technique can never be great.

Edmonds⁶¹ has described a method for studying colonic electrolyte transport in which a small amount of test solution, in a sac of dialysis tubing, is placed in the rectum or colon and changes in ion concentration with time observed. The technique has been well validated^{58, 61} and has the advantage of allowing clearly defined areas of the colon to be studied with

minimal disturbance to the subject. Because of the much smaller amounts of fluid used, it is a highly sensitive technique and very low doses of radioisotopes are needed for detailed studies. At the present time it seems to offer the best prospect of obtaining accurate information on short chain fatty acid transport.

Short chain fatty acid metabolism in the colon has been studied in a variety of other ways. These include faecal analysis,²⁰⁻²² the dialysis bag technique of Wrong,¹⁻¹⁹ direct installation of substrates into the caecum,²³ and *in vitro* fermentation studies, which are used widely in animal physiology. All of these should be considered when approaching a particular problem of colonic short chain fatty acid metabolism.

Clinical implications

ELECTROLYTE TRANSPORT AND DIARRHOEA

It is said that changes in human stool output are due to the effect of short chain fatty acids generated in the colon. These acids are thought of as poorly absorbed anions in man and to cause fluid retention in the colon by an osmotic effect. This view has been strengthened by the finding that, in children with diarrhoea due to carbohydrate malabsorption, stool output correlates with short chain fatty acid output²⁷⁻²⁹ and similarly in adults with diarrhoea due to malabsorption,³⁴ lactose intolerance,³⁵ and even catharsis with magnesium sulphate.² In some circumstances short chain fatty acids may even induce fluid secretion in the colon.³⁶ Other evidence for the laxative role of short chain fatty acid comes from a series of studies of the effect of dietary fibre on stool composition in man by Williams and Olmsted.³²⁻³⁷ They showed that stool weight correlated with short chain fatty acid output, and concluded that dietary fibre breakdown produced short chain fatty acids which, being unabsorbed, increased faecal weight.

However, it is now clear that short chain fatty acids are absorbed from the colon and that the increase in faecal weight with dietary fibre is due to physical effects of undigested fibre and bacterial mass. As colonic short chain fatty acid levels remain more or less constant despite dietary changes, etc, any factor increasing stool weight will increase output of these acids, even laxatives. The change in stool output in diarrhoea caused by carbohydrate malabsorption is now thought to be partly due to the osmotic effect of malabsorbed sugars.⁹⁸⁻¹⁰⁰ Furthermore, Argenzio¹⁰¹ has pointed out that short chain fatty acid production from glucose (equation 2) results in only a small increase in the theoretical osmotic pressure, as carbon dioxide is absorbed and bicarbonate reacts with short chain fatty acids as follows:

He also points out that these acids are the most rapidly absorbed ions in the colon and that their likely contribution to an increase in osmotic pressure will be small. In addition, acetate stimulates absorption of sodium and water from the colon at pH 6.4 and a case could be made for suggesting that absorption from the colon would be impaired in the absence of short chain fatty acids.⁷⁹

The case against these acids being involved in diarrhoea is strong. A possible effect could be through changes in pH. Where short chain fatty

acids are being generated rapidly the colonic buffering system may not be able to deal with production of acid and pH will fall. This may impair absorption of salt and water by the colonic mucosa¹⁰² and, in addition, as already described, promote lactate formation (a less well-absorbed anion) by the bacteria. Lactate may be important in the diarrhoea of childhood malabsorption, as significant quantities are found in the stools of these children. In general, however, it is difficult to implicate short chain fatty acids directly in either the control of faecal output or the genesis of diarrhoea in man.

ENERGY METABOLISM

The contribution of colonic fermentation in man to overall energy balance is unknown, but presents an intriguing problem. If, as was discussed earlier, the only substrate for fermentation in the colon is undigested dietary carbohydrate then about 20 g/day is broken down in man eating a United Kingdom type of diet. If we assume that about 70% of the potential energy is available for absorption as short chain fatty acids³² the net energy yield would be about 224 kJ (56 kcal), an insignificant amount. If, however, the energy requirement for growth and maintenance of bacteria in the colon are considered, then the potential energy available for absorption (as short chain fatty acids) would be 600–700 kJ or 7% of normal energy intake (assuming a 25% yield of bacterial cells from carbohydrate metabolised anaerobically). This represents considerably more carbohydrate breakdown than is currently believed to occur in the colon in subjects eating typical Western diets and needs to be further substantiated. The colon has not previously been thought of as a major factor in human energy metabolism. Much larger amounts of fermentable carbohydrate enter the colon of people living on high fibre diets, those with malabsorption, and in subjects who have had a jejunoileal bypass for obesity. Here short chain fatty acids may be making a significant contribution to energy balance.¹⁰

NITROGEN AND AMMONIA METABOLISM

Carbohydrate breakdown in the colon has important implications for nitrogen metabolism. When dietary fibre intakes increase in man faecal nitrogen excretion increases,^{19 103 104} an effect not seen with changes in protein intake.^{19 105} Faecal N was at one time thought to be either endogenous or 'metabolic' in origin (from gastrointestinal secretions and sloughed epithelial cells, etc) or from undigested dietary protein. It is clear from animal and human studies, however, that much of faecal nitrogen is present in bacteria^{106 107} and that when 'undigestible polysaccharides' are fed the increase in N excretion is due to an increase in faecal bacteria.¹⁰⁸ Similar findings have now been reported in man.¹⁰⁹

The main non-protein source of nitrogen in the colon is ammonia derived from the hydrolysis of urea. In the absence of active colonic fermentation much of this ammonia is reabsorbed to be reconverted to urea in the liver, a metabolic cycle which would seem to serve no useful purpose. Given a suitable energy source such as undigested carbohydrate,¹⁶ bacteria utilise ammonia nitrogen for protein synthesis³² and bacterial

growth is stimulated. In the horse¹¹⁰ the amount of protein nitrogen in the colon increases when the diet is supplemented with cellulose and ammonia concentration falls. Vince *et al.*¹¹¹ have shown, using human colonic microflora incubated anaerobically *in vitro*, that ammonia concentrations fall in the incubation system when an energy source such as lactulose is added, suggesting ammonia use for bacterial protein synthesis. In the rat,¹¹² blood urea is an important determinant of ammonia levels in the caecum and when plant cell-wall carbohydrate or lactose is added to caecal contents *in vivo* the production of short chain fatty acid increases and concurrently there is an increase in blood urea uptake, a fall in ammonia concentration in the caecum, and an increase in the portal arteriovenous difference in ammonia concentration. In patients with hepatic cirrhosis Weber¹¹³ observed urea production to fall and faecal N excretion to rise when his subjects were given lactulose, suggesting a reduction in ammonia recycling through the portal system and an increase in protein synthesis by the colonic microflora. All this evidence suggests therefore that microbial fermentation of carbohydrate reroutes nitrogen into bacterial protein synthesis and in so doing lowers colonic and portal venous blood ammonia levels.

Production of short chain fatty acids in the colon may also affect directly ammonia absorption. Carbohydrate fermentation lowers intraluminal pH,⁷² which would tend to trap ammonium ion in the lumen, as it is thought to cross the mucosa in the unionised (NH₃) form.^{114 115} Conversely, the effect of these acids on bicarbonate secretion would favour ammonia absorption by coupled non-ionic diffusion as suggested by Wrong.^{116 117} A direct study of the effect of short chain fatty acids on ammonia absorption would be worthwhile to try to clarify which of these possible interactions between fermentation and ammonia metabolism predominates in man.

Visek and his colleagues have pointed out^{118 121} that there could be other important implications of the relationship between the production of short chain fatty acids and ammonia metabolism in the colon. They have shown that ammonia can induce changes in intestinal cells which favour tumour growth. The generation of these acids in the colon by directing nitrogen metabolism towards bacterial protein synthesis, by lowering luminal ammonia levels, and by diminishing ammonia absorption would all reduce this risk. Such a possible protective effect for dietary fibre in the development of colon, and possibly other, malignancies, would fit current epidemiological findings.

MUCOSAL GROWTH AND DISEASE

There is little doubt that both the rumen epithelium and colonic mucosa metabolise short chain fatty acids, especially butyrate and propionate. In the rumen these acids have a stimulatory effect on epithelial growth.¹²² It is quite possible that they may exert a stimulatory effect on colonic mucosa as well. Recent studies¹²³ of diet on colonic mucosa in rats have shown that mucosal cell turnover declines during intravenous feeding and also when the same liquid diet is fed orally. A chow diet, however, which is rich in complex carbohydrate, stimulated cell turnover and increased colonic mucosal weight. This was attributed by the authors to the trophic

effect of gastrin, serum levels of which were higher with chow than with the liquid diet. When, however, the liquid diet was mixed with cellulose and fed, colonic mucosal growth was stimulated in the absence of a rise in serum gastrin, an effect which might well be ascribed to the presence of fermentation products.

In man the colonic mucosa is known to be dependent on butyrate as an energy source,¹²² particularly the distal colon. When Roediger¹²⁴ measured butyrate uptake in the colonic mucosal cells of patients with ulcerative colitis, he showed that it was reduced when compared with normal controls and suggested that failure to utilise butyrate could be a primary factor in the aetiology of ulcerative colitis. If this is the case, then it is equally possible that low butyrate production in the colon could precipitate colitis in susceptible individuals.

OTHER IMPLICATIONS

Short chain fatty acids are known to have an antibacterial effect.¹²⁵⁻¹²⁷ Production in the colon of these acids is one mechanism which prevents the establishment of pathogenic bacteria, such as salmonella species, although the low redox potential maintained by the predominantly anaerobic flora which fermentation supports may also contribute to this effect.

Other consequences of a lowering of pH include a favourable effect on vitamin K absorption,¹²⁸ the stimulation of mucus production,⁹⁶ and of magnesium absorption.¹²⁹

Conclusion

There are close parallels between the fermentative processes which go on in the rumen, caecum, and colon of herbivorous animals and colonic metabolism in man. Short chain fatty acids, which are the main end-product of carbohydrate breakdown in these organs, exert a controlling influence on intraluminal events, absorption, mucosal metabolism, and are accepted as such by animal physiologists.

Such recognition has yet to be given to this aspect of colonic function in man, and many studies of colonic metabolism have failed to take account of the possible effect of short chain fatty acids. A great deal still needs to be learnt about these acids in the human colon—in particular, the overall amount produced each day, the main substrates for fermentation, the effect of diet, the molecular form in which they are absorbed, their contribution to energy metabolism, and their interaction with a wide range of other colonic events. Such knowledge should yield important information which may be relevant to the aetiology of colonic disorders which are so prevalent in the human species.

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