PHYSIOLOGICAL REVIEWS
Vol. 81, No. 3, July 2001
Printed in U.S.A.

Short-Chain Fatty Acids and Human Colonic Function: Roles of Resistant Starch and Nonstarch Polysaccharides

DAVID L. TOPPING AND PETER M. CLIFTON

Commonwealth Scientific and Industrial Research Organization, Health Sciences and Nutrition, Adelaide, Australia

Topping, David L., and Peter M. Clifton. Short-Chain Fatty Acids and Human Colonic Function: Roles of Resistant Starch and Nonstarch Polysaccharides. *Physiol Rev* 81: 1031–1064, 2001.—Resistant starch (RS) is starch and products of its small intestinal digestion that enter the large bowel. It occurs for various reasons including chemical structure, cooking of food, chemical modification, and food mastication. Human colonic bacteria ferment RS and nonstarch polysaccharides (NSP; major components of dietary fiber) to short-chain fatty acids (SCFA), mainly acetate, propionate, and butyrate. SCFA stimulate colonic blood flow and fluid and electrolyte uptake. Butyrate is a preferred substrate for colonocytes and appears to promote a normal phenotype in these cells. Fermentation of some RS types favors butyrate production. Measurement of colonic fermentation in humans is difficult, and indirect measures (e.g., fecal samples) or animal models have been used. Of the latter, rodents appear to be of limited value, and pigs or dogs are preferable. RS is less effective than NSP in stool bulking, but epidemiological data suggest that it is more protective against colorectal cancer, possibly via butyrate. RS is a prebiotic, but knowledge of its other interactions with the microflora is limited. The contribution of RS to fermentation and colonic physiology seems to be greater than that of NSP. However, the lack of a generally accepted analytical procedure that accommodates the major influences on RS means this is yet to be established.

I. INTRODUCTION

Early observational studies showed that native East Africans, consuming a diet high in unrefined cereals, were at lower risk of colorectal cancer, diverticular disease, and constipation than Europeans who ate a diet low in such foods (45, 46). The early link to risk was with the overall diet that was high in starches, but attention became focussed on dietary fiber which was then thought to act as an indigestible bulking agent. Fiber is comprised principally of polysaccharides (nonstarch polysaccharides, NSP), and it has been established that, depending on type, they are subject to varying degrees of breakdown on transit in humans. This is effected in the human large bowel by a complex bacterial ecosystem resembling that found in obligate herbivores. It has similar substrates, i.e., complex carbohydrates and end products (short-chain fatty acids, SCFA), mainly acetate, propionate, and butyrate. SCFA contribute to normal large bowel function and prevent pathology through their actions in the lumen and on the colonic musculature and vasculature and through their metabolism by colonocytes. Butyrate, in particular, is thought to play a role in maintaining a normal colonocyte population. In ruminants and other herbivores, SCFA are absorbed and transported via the portal vein to the liver, and the fraction not absorbed is distributed to the other body organs and tissues for metabolism (for a general review, see Ref. 28). In herbivores, peripheral venous SCFA concentrations are high due to comparatively low visceral extraction and high rates of absorption into the circulation. However, human peripheral venous blood concentrations are normally low, and only acetate is present in measurable amounts. This profile reflects the lower SCFA production rates and greater visceral extraction in omnivores, meaning that human peripheral venous SCFA are not representative of those in the portal circulation. Human experimentation has been confined largely to fecal measurements, which are also limited as >95% of SCFA are produced and absorbed within the colon. Breath H_2 evolution has been used but is extremely limited in value as gas production is not indicative of the SCFA that are produced. Incubation of fermentable carbohydrates with fecal inocula can provide valuable information provided a number of precautions are taken, especially in minimizing donor variability. SCFA in colonic contents have been determined in colostomy patients and post mortem, but these approaches are impractical for large-scale dietary studies. Fecal measures are useful in establishing changes in excretion but not necessarily in production because fecal SCFA can be influenced by rate of transit alone. Consequently, most experimental data have been obtained from model systems. Animal studies, principally with rats and pigs, have shown that large bowel SCFA are increased by the provision of fermentable carbohydrates. However, rats are coprophagic and the large bowel differs substantially from humans, and pigs (and possibly dogs) appear to be better models.

Although NSP resist digestion by intrinsic human intestinal digestive enzymes completely, their intakes do not account for calculated human SCFA production (the "carbohydrate gap"). Some of the deficit may be filled by oligosaccharides (OS), but starch and products of small intestinal starch digestion are thought to contribute the most. This fraction is termed resistant starch (RS). This review aims to examine the relative contributions of RS and NSP to SCFA production in the context of the epidemiological and other data linking complex carbohydrates to improved colon function and lowered disease risk. In view of the reliance placed on animal models and indirect measures of fermentation, the strengths and limitations of these experimental approaches will be evaluated concurrently. A particular problem is that assay procedures are well-established for fiber and/or NSP but not for RS. This means that dietary intakes can be calculated for the former but not the latter, and direct comparison of effects in the body may be difficult. Thus health authorities have been able to make dietary recommendations for fiber but not yet for RS (22). In this review, the primary focus is on adults, but some attention is given to infants. The fermentative products in preweaned infants differ considerably from adults, with little butyrate being found. Other acids (e.g., formate) and products (e.g., ethanol) are found in substantial quantities but not in adults. The relationship between weaning to solid foods, the microflora, and products of fermentation remains to be elucidated. In adults, the production of individual SCFA in the colon is important as is their distribution along the large bowel. Fermentation predominates in the proximal colon and SCFA transported to distal regions by the fecal stream. Samples from patients with colostomy at various sites support a decline in SCFA levels along the large bowel. The distal colon is the site of greatest organic disease, so the delivery of butyrate to this viscus may be especially important. This distribution of SCFA along the colon is found in other omnivores (e.g., pigs) but not in rodents.

The fiber hypothesis led to expectations of a strong protective role for it in laxation, and this is well-documented, especially for insoluble fiber. This is not so for colorectal cancer, an important malignancy in affluent countries. Experimental work (largely in rats) suggested strong protection by fiber against chemically induced large bowel tumors. In contrast, epidemiological studies showed that any protection was weak. Interventions, where human volunteers with polyps or adenomas have consumed fiber supplements (usually as cereal brans), have also yielded disappointing data on progression or recurrence. Conversely, studies in rats have indicated no beneficial or adverse effects of RS on tumors in genetically or chemically induced tumors, while epidemiologi-

cal studies show that starch and (projected) RS intakes correlate negatively with colorectal cancer risk. Human interventions have shown that greater RS consumption is associated with diminished risk as shown by various indices (e.g., SCFA, colonocyte proliferation). The paradox may accrue from particular features of rodent digestion (e.g., coprophagy) which limits the value of the data. In vivo and in vitro studies indicate that butyrate produced by RS fermentation may be protective (possibly by promoting apoptosis in tumor cells), but direct proof of such a role for RS in colorectal cancer is absent. RS has been shown to promote colon function by alleviating infectious diarrhea and promoting colonic mineral absorption. Part of the benefits in diarrhea may be due to interactions between RS and the microflora. One RS type (high amylose maize starch) has been shown to be a prebiotic and promotes the survival of lactic acid bacteria. The interactions of RS with the microflora in general remain to be elucidated. Although RS could be regarded as a malabsorbed carbohydrate, there is little evidence of any deleterious effect of RS in humans; rather, it appears that interactions between RS and the colonic microflora appear to be of benefit to the host in the short and long term. In countries with low intakes of starch and RS, it may be of health benefit to increase their intakes, but it is not clear whether it should be as total starch, RS, or starch and RS.

II. MODES OF ACTION OF FIBER IN THE GASTROINTESTINAL TRACT

The first systematic links between dietary fiber and human health were expressed in terms of its indigestibility (45, 46). Although breakdown of some fiber components on passage through the gut was recognized, it was considered largely as a bulking agent (45) and was defined as "plant structural and exudative components not digested by human digestive enzymes" (275). This has been called the "roughage model" whereby any protection by fiber was due to its dilution or binding of toxins and carcinogens in the intestines through its physical presence (302). The then-current analytical procedures accorded with that concept, with gravimetric measurement of residues after extraction of foods or ingredients with neutral or acid detergent solutions yielding a residue of insoluble fiber components (241). Application of this, rather limited, technology to humans showed that the breakdown of fiber on transit in humans was surprisingly large for some foods. For example, during passage of wheat bran, only 36% was degraded but only 8% of cabbage fiber survived (282). The neutral detergent fiber (NDF) procedure used in that study is a substantial underestimate as soluble material, including important fiber components, are extracted and so not included in the fiber value (241). Comprehensive analyses have been developed for the major fiber components that obviate such losses (e.g., Ref. 293), and their application shows that fiber in the human diet is principally NSP and Klason lignin, an insoluble, noncarbohydrate residue (275). Rapid enzymatic-gravimetric methods, involving digestion of foods with enzymes to remove digestible components including starch, protein, and fat, have been developed. One of these methods has been validated and accepted by the Association of Official Analytical Chemists and Food and Agricultural and World Health Organizations (for details of the variants, see Ref. 17). This procedure yields values termed total dietary fiber (TDF). There are other components such as oligosaccharides that are also not measured routinely. These can be regarded as components of dietary fiber through their indigestibility, but their exact dietary contribution is unknown (274). The contribution of lignin to the diet may be as low as <1 g/day (182), compared with 15–20+ g/day for NSP (22). Thus NSP could be taken as the principal contributors to dietary fiber intakes, as has been done for the purposes of this review. NSP can be subdivided into soluble and insoluble NSP, based on their solubility in aqueous solutions, although not necessarily under physiological conditions (299). It appears that foods high in soluble NSP undergo the greatest losses on transit (302). These losses are accompanied by a greater fecal excretion of bacteria, which increased by 2.3 g/day with wheat bran and 4.8 g/day with cabbage (282). The bacteria are derived from the organisms resident in the human large bowel. It is they which degrade carbohydrates entering the large bowel, a process which has a direct impact on colonic function.

III. LARGE BOWEL MICROFLORA, FERMENTATION, AND SHORT-CHAIN FATTY ACID PRODUCTION

A. Large Bowel Microflora

The bacterial population of the human cecum and colon is numerically large with at least 10^{10} to 10^{11} cfu/g wet wt, which, with an estimated mass of 250–750 g of digesta, gives a calculated total of $\sim 10^{13}$ cfu in the whole hindgut (138). Similar values have been reported in other omnivores such as pigs (47). Bacteria comprise ~40–55% of solid stool matter (77), and ~15 g of fecal bacterial biomass is voided daily in individuals consuming "Western-type" diets (138). More than 50 genera and over 400 species of bacteria have been identified in human feces (100, 120, 138, 258). The dominant organisms in terms of numbers are anaerobes including bacteroides, bifidobacteria, eubacteria, streptococci, and lactobacilli, while others, such as enterobacteria, also may be found, usually in

fewer numbers. Generally, bacteroides (including those that can utilize a wide range of polysaccharides) are most numerous and can comprise more than 30% of the total. The microflora can metabolize proteins and protein degradation products, sulfur-containing compounds, and endogenous and exogenous glycoproteins (120). Some organisms grow on intermediate products of fermentation such as H_2, lactate, succinate, formate, and ethanol and convert these to end products including SCFA (177). Other organisms metabolize CO_2 either yielding CH_4 (199) or converting CO_2 to acetate (84). Breath CH_4 excretion reflects methanogenic bacterial activity in the colon (227) but occurs only in individuals colonized by a particular organism (*Methanobrevibacter smithii*) at $>10^8$ cfu/g dry feces (199). Bacterial numbers, fermentation, and proliferation are greatest in the proximal large bowel where substrates are highest. These substrates are depleted on transit, which is reflected by a decline in SCFA production (177, 178). In vitro endogenous production was ~250 mmol SCFA·kg^{-1}·48 h^{-1} during incubation with proximal colonic inocula falling to ~50 mmol SCFA·kg^{-1}·48 h^{-1} with distal colonic inocula (177). There may be population (as well as numerical) changes on transit due to changes in substrate supply (258).

The intestines of humans and other animals are sterile in utero with colonization by maternal anal or vaginal organisms occurring during birth (197). Colonization is time dependent, with enterobacteria and streptococci predominating during the first 1–3 days after birth when fecal concentrations of these organisms peak at ~10^{11} cfu/g feces before declining (201). Bifidobacteria appear in feces after 2 or more days after birth and become the dominant species at ~4–5 days. Colonization by bifidobacteria is significantly higher in breast-fed babies (47.6% of babies vs. 15% fed by bottle) (250), whereas enterococci predominate in bottle fed infants (7.4 vs. 6.7 $\log_{10}$ cfu/g feces in breast fed infants). On weaning, bifidobacteria decrease and a more "adult" profile develops, presumably reflecting dietary change (201). The relationship of dietary NSP and RS to this process is unknown.

The colonic microflora should change in response to gross nutritional shifts (e.g., weaning), progressive change (such as aging), or variations in food intake. In aged persons, *Escherichia coli*, streptococci, and clostridia increase and bifidobacteria decrease further (201). Some studies have linked increasing age with the number of people colonized by methanogens (129) and their activity (as measured by breath CH_4 evolution) (99), but other studies have not (33, 194), suggesting that any association may be weak. Very little is known of the role of heredity. A study of two genetically distinct strains of pig (Chinese and United States domestic) showed that the diet was the primary determinant of the effects of fiber on large bowel microflora and SCFA (186). Information in these areas is limited because conventional microbiological techniques are very labor intensive. Newer molecular biological techniques should make investigation easier, quicker, and more discriminating (290). These technologies also raise some concerns about the reliability of traditional methodologies. In a continuous culture of human feces, plating methods showed that at 21 days, >98% of the total culturable count was bifidobacteria and lactobacilli, whereas with genus-specific 16S rRNA oligonucleotide probes, bifidobacteria were absent and lactobacilli represented ~25% of total 16S rRNA at the same time point (266). The methodological issues in bacterial enumeration are hampering the understanding of relationships between substrate supply, fermentation, and end products. Until they are resolved, indirect indices of bacterial activity (e.g., breath gas evolution or the production of specific SCFA in vitro or in animal models) will remain in widespread use because they provide measures of the metabolic products that actually modulate physiological changes.

B. Fermentation and Large Bowel SCFA

The basic fermentative reaction in the human colon is similar to that in obligate herbivores: hydrolysis of polysaccharides, oligosaccharides, and disaccharides to their constituent sugars, which are then fermented resulting in an increased biomass (258). Carbohydrate hydrolysis is effected by a number of bacterial cell-associated and secreted hydrolases that can digest a range of carbohydrates which the human host cannot. Fermentation yields metabolizable energy for microbial growth and maintenance and also metabolic end products. Nitrogen for protein synthesis can come either from urea (via the urease reaction), undigested dietary protein, or endogenous secretions. In adult humans, the principal products are SCFA together with gases (CO_2, CH_4, and H_2) and some heat. The general reaction of SCFA production and overall stoichiometry has been summarized for a hexose (73) as follows: 59 $C_6H_{12}O_6$ + 38 H_2O → 60 CH_3COOH + 22 CH_3CH_2COOH + 18 CH_3CH_2 CH_2 $COOH$ + 96 CO_2 + 268 H^+ + heat + additional bacteria.

The balance of products differs for other substrates (e.g., uronic acids and pentoses) but is expected to be generally similar (175). Survey data from various populations show that fecal SCFA are in the order predicted from that equation, i.e., acetate > propionate ≥ butyrate (77, 103, 144, 211, 265, 289) (Table 1). Other organic acids (e.g., lactate or succinate or branched-chain SCFA generated from amino acids) are found in much smaller amounts. In milk-fed infants, acetate is the major acid in feces. Propionate levels are very low while butyrate is virtually absent in babies fed breast milk but may be found in those fed formula (88, 270) (Table 1). No lactate was found in formula-fed infants, but 13.9 mmol lactate/kg

TABLE 1. *Fiber intakes and fecal SCFA in adult and infant populations*

Study	Population and Diet	Fiber	Acetate	Propionate	Butyrate
		(g/day)		(mg/g wet feces)	
Adults					
Fleming et al. (103)	US; self selected, low and high fiber	33*	2.75	1.22	1.64
				(mM)	
Muir et al. (211)	Simulated Australian	24†	56.1	15.0	18.4
	Simulated Chinese	14	39.9	12.8	12.2
Takahashi et al. (289)‡	Japanese; self selected	NR	36.0	22.3	17.5
	Japanese; controlled	21.7	45.2	23.6	19.0
				(mmol/kg wet feces)	
Hoverstad and Bjorneklett (144)	Norwegian; self selected	NR	37.3	12.5	12.4
Segal et al. (265)	South African blacks	13.5§	83.6	32.3	17.9
	South African whites	NR	40.5	11.9	12.4
				(mmol/kg wet feces)	
Infants					
Edwards et al. (88)	Breast (4 wk)	NA	46.8	1.2	0.0
	Formula (4 wk)		43.6	13.4	2.2
Siigur et al. (270)	Breast (2 mo)	NA	53.9	2.9	0.4
	Formula (2 mo)		52.6	16.2	2.2

NR, not recorded; NA, not applicable. * As total dietary fiber. † As nonstarch polysaccharides plus lignin. ‡ Calculated from fecal water values and daily short-chain fatty acid (SCFA) output. § As dietary fiber.

of feces was found in breast-fed babies. Formate and ethanol have been found in quantity in feces from breast-fed babies (333). The SCFA profile may be important in gut development. Data from premature infants (which are maintained in incubators) suggest that there is a very sensitive period between *days 14* and *21* of life when fecal butyrate increases by 300%, and its excessive production (or the organisms which produce it) may relate to the development of necrotizing enterocolitis which is a substantial threat in these infants (288). It appears that in healthy infants, fermentation is slower than in adults, and butyrate production is established more slowly than that of acetate and propionate but by 2 years an adult SCFA profile has emerged (198). Presumably, the product profile during milk feeding contributes to the specific metabolic needs at this period of development, but this remains to be established as do the changes in individual SCFA during weaning and maturation.

C. Measurement of Large Bowel SCFA in Humans

Intubation has been used to determine the intestinal digestibility of carbohydrates (including starch) in humans (56, 97, 283) but not yet for SCFA. SCFA have been determined in human gut contents and portal venous blood at autopsy (78) and in portal venous blood of patients during surgery (76, 79, 224, 272). Clearly, these approaches are limited. Dialysis sacs in gelatin capsules have been used to determine SCFA in situ in normal subjects (335) and in dietary interventions with different types of fiber (110). They have been used clinically in ulcerative colitis (245) where the severity of inflammation correlated with high concentrations of butyrate (18.9 vs. 14 mM in controls) and lower pH (6.21 vs. 7.47 in controls) in the patients affected most. Continuous sampling with this method is impractical, and the relationship between transit and SCFA is unclear. SCFA have been measured in the stomal effluent of patients with ileostomy, transverse, or sigmoid colostomy and who were consuming a self-selected diet (200). SCFA excretion was high with transverse colostomy compared with sigmoid colostomy, which is consistent with the expected fall in fermentation on transit.

1. Regional considerations of colonic SCFA metabolism

Elsden et al. (90) showed both high concentrations of and a progressive decline in volatile acid along the large bowel of a number of herbivorous and omnivorous animal species. The profile has been confirmed in pigs where the fall can be substantial (20, 32, 78, 124, 183–185, 221, 303). Depending on diet, total SCFA concentrations in the proximal colon are ~70–140 mM falling to 20–70 mM in the distal colon (Table 2). Neither total SCFA nor the individual acids in the distal colon are predictive of those found proximally (32, 183, 184, 303). Fecal values have been measured but not at the time of sampling of gut contents and show increases in the excretion of total and individ-

TABLE 2. *Total SCFA and butyrate (in parentheses) in regions of the large bowel of pigs fed various sources of fiber and resistant starch*

Study	Carbohydrate Sources	Fiber	Large Bowel Region			
			Cecum	Proximal colon	Mid colon	Distal colon
		(g/day)	(mmol/kg contents)			
Bach-Knudsen et al. (19)*	Wheat flour	62†	100	90	60	60
	Rolled oats + oat bran	194	145	125	90	60
Cummings et al. (78)	Pig grower diet	NR	83	NR	114	70
			(mM)			
Fleming et al. (102)	Beans	63‡	110 (9)	NR	NR	NR
	Bran	66	140 (11)	NR	NR	NR
		(g/kg diet)	(mmol/kg contents)			
Glitso et al. (124)	Whole rye	156†	156	123	70	54
	Rye pericarp	177	112	107	77	70
	Rye aleurone	180	164	160	122	74
	Rye endosperm	94	151	104	82	57
		(g/day)	(mM)			
Marsono et al. (184)	White rice	20†	82 (8)	78 (6)	36 (3)	25 (2)
	White rice + rice bran	43	69 (5)	77 (8)	52 (6)	30 (4)
	Brown rice	37	72 (5)	96 (9)	87 (10)	65 (8)
Bird et al. (32)	White rice + rice bran	33†	69 (4)	43 (4)	32 (72)	21 (2)
	Brown rice	33	63 (4)	81 (6)	72 (8)	63 (9)
Topping et al. (303)	Low fiber, wheat bran	14†	70 (8)	66 (9)	50 (7)	19 (2)
	Wheat bran	44	131 (13)	94 (12)	85 (12)	52 (5)
	Beans	45	124 (6)	139 (9)	80 (7)	65 (6)
	Oat bran	42	92 (8)	97 (12)	73 (10)	39 (5)
		(g/day)	(mmol/kg dry matter)			
Stanogias and Pearce (277)	Wheat bran	75§	NR	181	NR	NR
		150	NR	356	NR	NR
		300	NR	458	NR	NR

The values for "fiber" refer either to the amount fed to each animal per day (g/day) or the amount incorporated into each diet (g/kg diet). NR, not recorded. * Values by interpolation from graphical data. † As nonstarch polysaccharides plus lignin. ‡ As total dietary fiber. § As neutral detergent fiber.

ual acids in pigs fed high RS diets (32, 43, 301). SCFA availability in the distal colon can change on transit with the loss of water and digesta mass. For example, in pigs fed beans and a low-fiber control diet, respective digesta masses were 198 and 103 g in the proximal colon and 30 and 21 g in the distal colon. Corresponding SCFA pools were 22.6 and 5.35 mmol and 1.43 and 0.23 mmol, respectively (303). The relative change was greatest for butyrate. In pigs fed white rice (low RS), the distal colonic butyrate pool was 0.06 mmol compared 0.47 mmol in pigs fed brown rice (high RS) (183). SCFA availability changes with rate of digesta passage independently of rates of production. When humans were given senna or wheat bran, transit was 39 or 41 h, respectively, compared with 74 h with loperamide. Mean total fecal SCFA and butyrate concentrations were 113 and 79 μmol/g wet wt (wheat bran), 202 and 59 μmol/g wet wt (senna), and 82 and 6 μmol/g wet wt (loperamide), respectively (170). There is a curvilinear relationship between transit and fecal total and individual SCFA (especially butyrate) so that at whole gut transit times >50 h, butyrate cannot be detected (probably due to colonic uptake). This is an additional variable to be considered when analyzing fecal values, especially when some studies have shown greater fermentation (as breath H_2 evolution) with consumption of fermentable carbohydrate but no change in fecal variables. Tomlin and Read (298) raised the RS intake (as a breakfast cereal) of human volunteers from 0.86 to 10.3 g/day. Integrated breath H_2 production measured over 8 h was raised significantly from 7,529 to 12,072 ppm/min but fecal SCFA were unchanged, suggesting that any change was localized within the colon. The data from stomal patients (200) provide direct support for an SCFA gradient in humans. Concentrations in sigmoid colostomy fluid and feces were ~40–50% of those in patients with transverse colostomy. This fall is much larger than in postmortem samples where total SCFA values were 118.6, 105.4, 72.4, and 87.5 mmol/kg in the ascending, transverse, descending, and sigmoid colon/rectum, respectively (78).

Regional differences in SCFA have implications for

large bowel disease, especially cancer (54), which is an important malignancy in terms of numbers affected, particularly in affluent westernized societies (334). In these populations tumors predominate distally with incidence rates of 9.5 and 7.3/100,000 of population, for American men and women, respectively, 8–15 cm from the rectum compared with 2.8 and 3.8 in the ascending colon (70). Other conditions (e.g., ulcerative colitis) where SCFA may have a role also predominate in the distal colon. This means that there are several important questions in human large bowel fermentation: *1*) Does the overall rate change with diet? *2*) Is the production of individual SCFA altered? *3*) What is the distribution of the resulting SCFA along the colon?

2. *Methods for assessing fermentation and SCFA production in humans*

Stable isotope technology, in which labeled carbohydrates are consumed and metabolites monitored in blood or expired air, has been applied in a very limited way to SCFA production in humans and pigs (73). It has yet to be tested thoroughly in human dietary studies. The other techniques in current use in vivo are measurement of breath gas (H_2 or CH_4) or SCFA in peripheral venous plasma or feces. In vitro SCFA production can be measured using fecal or digesta homogenates, but its relationship to the situation in vivo is equivocal. Breath gas evolution is noninvasive and can be carried out in real time and has been shown to increase under conditions favoring fermentation. Gelissen et al. (117) fed subjects with low (2.6 g) and high (15.7 g) fiber test meals and found that evolution was 158 and 167 ppm H_2/h on *days 3* and *5* of consumption of the low-fiber meals and 492 and 554 ppm H_2/h with the high-fiber meals. Acarbose is a potent α-glucosidase inhibitor. It is a pseudo-oligosaccharide consisting of an unsaturated aminocyclitol, a deoxyhexose, and a maltose (for graphical structure, see Ref. 39). Inhibition of small intestinal starch digestion through ingesting this agent raises breath H_2 excretion and fecal SCFA excretion in humans (142, 259, 260, 326). Scheppach et al. (259) showed that breath H_2 was ~81 ppm for a test meal with acarbose compared with a maximum of 32 ppm for the test meal alone. Stool weight rose by 68% with the inhibitor. Weaver et al. (326) noted that bowel movements were increased from 8–9/wk when subjects consumed the placebo to 16/wk at a dose of 200 mg acarbose. Total SCFA excretion with and without the inhibitor was 14.8 and 7.6 mmol/day, respectively (260). Holt et al. (142) reported that these effects persist for up to 1 yr of acarbose treatment. Other feeding trials with RS (230, 313) have shown greater breath H_2 evolution with consumption of fermentable carbohydrate. van Munster et al. (313) showed an increment of excretion from 101 to 186 ppm H_2/h when subjects consumed an additional 45 g of high amylose starch. SCFA excretion rose from 7.1 to 9.6 mmol/day. However, the technology is limited by the fact that some individuals do not produce H_2 (117), so a stoichiometric relationship between gas evolution and production of total and individual SCFA is impossible. Consumption of some fermentable carbohydrates such as transgalacto-oligosaccharides (36) lowers breath H_2 despite in vitro evidence of greater SCFA production. In subjects consuming 10 g of this product for 21 days, CH_4 production stayed constant at ~800 ml/12 h, whereas H_2 values fell from 476 ml/12 h on *day 1* to 164, 267, and 206 ml/12 h on *days 7*, *14*, and *21*, respectively. Flick and Perman (104) found breath H_2 evolution was unchanged in volunteers consuming 40 g of lactulose/day for 1 wk despite evidence in vitro of greater SCFA production through lower pH values with fecal inocula. Breath H_2 measurement is relatively easy to use but appears to be rather unreliable and incapable of further development.

Peripheral venous blood acetate has been used to monitor large bowel events, but there are only a few published reports of its use. Pomare et al. (229) showed a rapid (<90 min) rise and fall in mixed venous acetate after oral consumption of a solution of 50 mmol SCFA (30 mmol acetate and 10 mmol each of propionate and butyrate). This time course is similar to that seen in pigs fed sodium propionate and is consistent with absorption from the stomach (147). The maximum concentration achieved was 194 μM against a baseline of 54 μM (229), which is in the range noted by Muir et al. (209) and Wolever et al. (332). Increments are slower and more sustained after ingestion of lactulose or pectin, consistent with SCFA production by large bowel fermentation (229). A rise in portal venous SCFA (including propionate and butyrate) was reported in patients at surgery after fermentation was increased by cecal installation of lactulose (224). Peak concentrations after infusion of 10 g of lactulose were 0.24, 0.04, and 0.03 mM for acetate, propionate, and butyrate, respectively. These values are low compared with those recorded in rats and pigs where total portal venous SCFA can exceed 2 mM (e.g., Refs. 60, 147) and may reflect the small amount of lactulose that was given. Acetate is the main SCFA in mixed venous blood, and propionate and butyrate concentrations are so low that measurement is difficult without considerable sample concentration (209, 332). Wolever et al. (332) recorded values of 4.5–6.6 and 2.0–3.9 μM for propionate and butyrate, respectively. Corresponding values reported by Muir et al. (209) were higher at 17.3–32.8 and 36.3–65.5 μM, respectively. Blood acetate alone is of little value as an indicator of SCFA, especially if the other acids are important metabolically. Data from blood-perfused liver (273) and heart (306) show that both organs buffer blood acetate with uptake above a concentration of ~0.25 mM and net release below it. Similar hepatic buffering and equilibrium point have been reported in rats in vivo (44)

and may occur in humans (262, 272) and would limit the value of changes in blood acetate greatly. Peripheral venous SCFA seem to resemble breath gases; they are general indicators of fermentation but not of changes within the viscera.

In vitro fermentation of forage with rumen liquor has been used very successfully to determine its nutritional value for ruminant domestic animals (327). SCFA production from foods and ingredients by human fecal homogenates has been examined in a similar manner. The method has the advantage of avoiding complications due to uptake and utilization by colonocytes. Generally, batch cultures (where inocula are incubated with substrate in bottles) have been used. However, the wide range of incubation times, fecal inocula strength, substrate concentration, buffering of medium, addition of protein and micronutrients, and analytical procedures for the substrate make direct comparison between studies difficult. The inoculum itself can be a major factor with considerable time-dependent variability between donors when the same substrates are fermented (87, 203, 205, 258). For example, control production on 1 day of sampling ranged from 20 to 42 mM/24 h (205). In the same study, production from wheat bran by three subjects measured on three separate days ranged from 59 to 111 mM/24 h. Some subjects (possibly >20% of those sampled) seem not to metabolize substrates such as particular types of RS well in vivo (64, 74) or in vitro (64). To minimize this potential variability, McBurney and Thompson (189) recommended use of a minimum of three donors. Other technical issues, e.g., maintenance of a reducing environment, buffering the medium against a fall in pH (87), and the dilution of the inocula (25), can influence SCFA production. Protein may have an influence as it appears to be fermented very rapidly by batch cultures (205). Animal studies suggest that resistant protein (RP, named by analogy with RS) can be an important experimental variable (202). In rats fed a diet containing a highly digestible protein (casein) and 200 g/kg of a high amylose cornstarch, succinate and butyrate were present at 651 and 26 μmol/cecum, respectively. Partial replacement of casein with an RP (autoclaved egg white) lowered succinate to 381 μmol/cecum and raised butyrate to 111 μmol/cecum. Substrate concentration is important and varies with fiber source. Mortensen et al. (203) showed that the molar proportion of butyrate rose from 9 to 20% by increasing the concentration of pectin from 2.5 to 30 mg/ml. With isphagula, the molar proportion of butyrate rose from 8 to 11%. One large European collaborative study involving five centers has evaluated fermentation under standardized conditions of incubation and NSP analysis (25). This offers promise of making direct comparison between studies easier. However, the technique remains an intrinsically limited means of studying dietary influences on SCFA production.

Changes in SCFA excretion with diet have been examined in colostomates (4, 222). Pant et al. (222) found that wheat bran raised SCFA excretion, whereas it was lowered by consumption of oat bran. However, the feeding time in this study was very short (5 days). Ahmed et al. (4) compared SCFA excretion with a high and low RS diet and found that it was significantly higher with the former (183 vs. 116 mmol/kg dry fecal wt). These limited data confirm that human colonic SCFA seem to respond to change in fermentable substrate as they do in model animal species.

3. Animal models for human large bowel SCFA metabolism

A priori, the model species should be as close to humans as possible, i.e., omnivorous with appropriate food intakes, nutrient requirements, and gastrointestinal system. The dog appears to be particularly suitable (315) because its large bowel contributes 14% to total digestive tract volume compared with 17% in humans, 48% in pigs, and 61% in rats. Relationships between fermentable carbohydrates and SCFA have been studied in intact (e.g., Ref. 287) and surgically modified dogs (212) for the purpose of improving canine, not human, nutrition. Compared with pigs, dogs have found relatively little use, probably for social reasons. The pig appears to be optimal especially when considering such issues and the fact that it consumes human foods readily. The relatively large fractional volume of the porcine colon (due to greater length, rather than cross-sectional area) necessitates intakes of dietary fiber (80–100 g/day for a 60-kg animal) to maintain laxation, which are higher than those for humans but do not appear to compromise the data. Pigs have been used to examine the effects of numerous human foods and food ingredients including beans (102, 303), rye (124), rice (31, 184), oats (21, 303), starches (185, 301), tagatose (163), and wheat bran (20, 277, 303) and its fractions (20) on large bowel SCFA. All of these studies showed greater large bowel SCFA after consumption of fermentable carbohydrates (Table 2). By implication, SCFA production was increased. In pigs with cecal or proximal colon cannulae, SCFA were increased by feeding of navy beans (102) or wheat bran (277). Electron microscopic examination of native starch granules (Fig. 1*A*) and those recovered in human ileostomy effluent (Fig. 1*B*) and porcine cecal contents (Fig. 1*C*) showed substantial pitting and etching (301). However, the patterns were similar in both digesta samples, and these and the other experimental data suggest a good degree of similarity between pigs and humans. Pigs are also useful models for clinical conditions such as infant necrotizing enterocolitis (83) but not for colon carcinoma. Injection of carcinogens such as dimethylhydrazine (DMH) or azoxymethane (AOM) into rats induces intestinal cancers that can be

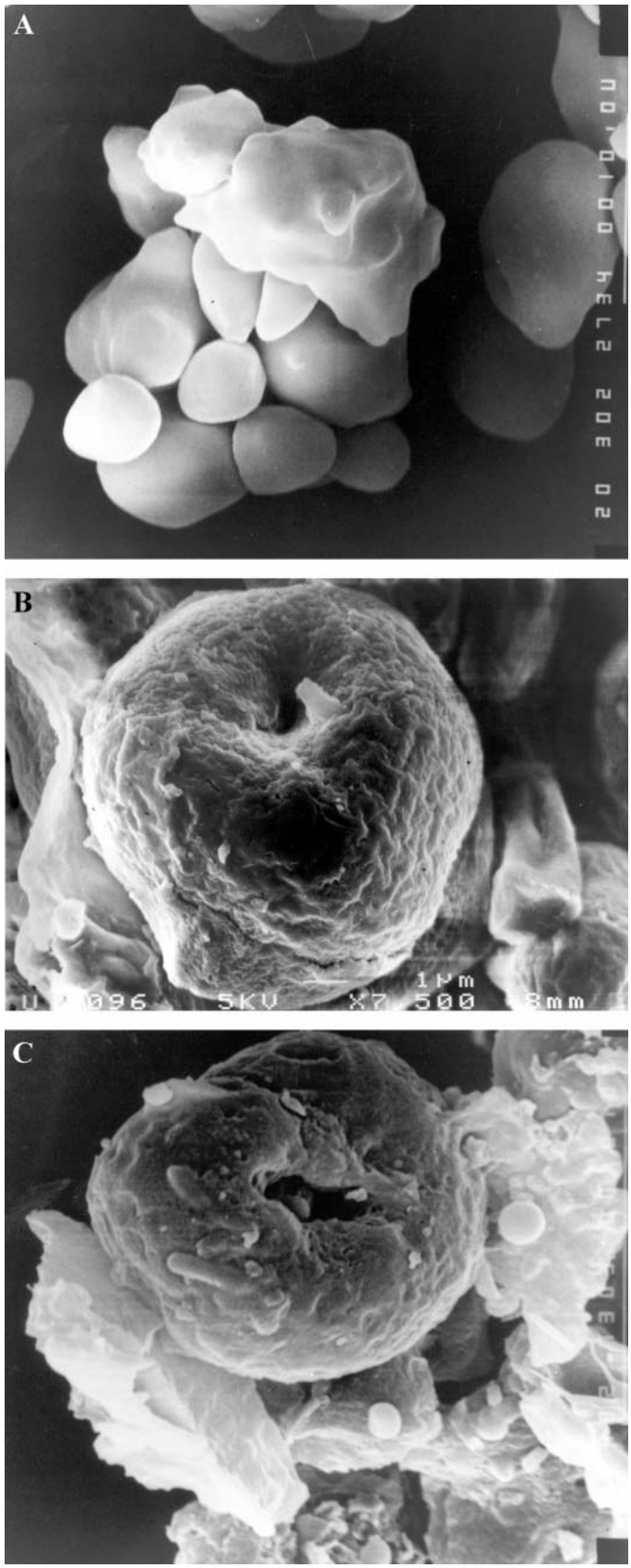

TABLE 3. *Prevention of coprophagy and cecal SCFA in rats fed a control (nonpurified) diet or diets containing oat or wheat bran*

Diet	Coprophagy	Total SCFA, mM	Butyrate, mM
Nonpurified	Yes	188	51
	No	101	29
Oat bran	Yes	224	37
	No	197	6
Wheat bran	Yes	48	48
	No	33	33

[Modified from Jackson and Topping (149).]

modified by diet, but in pigs DMH produces hepatic necrosis without any intestinal tumors (330). There is no porcine equivalent of rodent models such as the multiple intestinal neoplasms (Min) mouse (208) or Smad3 mutant mouse (345) that have a genetic predisposition to intestinal cancer. These limitations plus the relative cheapness, small size, and ready availability of rodents explains their wide experimental use. However, it overlooks the fact that (unlike pigs and humans) rats are coprophagic cecal fermenters with a complex musculature that ensures selective retention of liquid digesta in that viscus while solid material is voided (284, 315). Rats reingest the feces produced by cecal fermentation specifically. This has very important implications for the digestion of RS but not necessarily of fiber. The fermentation of insoluble fiber differs little between rats in which coprophagy is allowed or not (71), and losses of neutral NSP in wheat bran, apple, cabbage, and carrot are similar in humans and rats (216). In contrast, 17% of starch in flaked barley appeared to resist small intestinal digestion in humans but only 1% in rats (242). In these studies , the starch-to-NSP ratio was 0.89 in humans and 0.02 in unrestrained rats (where coprophagy was allowed). Only one study seems to have examined abolition of coprophagy and SCFA where cecal values were changed substantially, depending on fiber source (149) (Table 3). Coprophagy is a very important variable, especially for RS and SCFA, and limits the value of rat data. It seems preferable to use studies in humans wherever possible and, failing that, use more suitable species such as pigs or dogs.

FIG. 1. Scanning electron micrographs of native high-amylose starch granules (*A*) and granules isolated from human ileostomy effluent (*B*) and the large bowel of a pig with a cannula inserted into the cecum and proximal large bowel (*C*). Native (ungelatinized) granules of a high-amylose (70% of total) starch were examined by scanning electron microscopy and show the characteristic irregular shape of these granules. After passage through the small intestine, the granules were recovered from the terminal human ileum and proximal porcine large bowel. They have undergone amylolysis by small intestinal α-amylase, and both exhibit similar patterns of etching and pitting. The starch residue remaining after amylolysis is believed to be high in amylose and is known to be fermented by the large bowel microflora. [Modified from Brown et al. (43).]

IV. METABOLIC EFFECTS OF SHORT-CHAIN FATTY ACIDS IN THE LARGE BOWEL

Carbohydrates entering the large bowel can alter colonic physiology in two ways: physical presence and fermentation. Undigested mono-, di-, and oligosaccharides induce osmotic diarrhea if consumed to excess (73). Fecal bulking was an important component of the fiber hypothesis (302) and is a recognized attribute of foods such as cereal brans (23, 116, 152, 164, 165). Fiber analogs such as plastic "bran" flakes also speed transit and promote laxation (171), indicating the importance of the roughage effect of fiber. The actions of SCFA are wider in scope and more significant to the colon, and this review focuses on the major acids found in adults, acetate, propionate, and butyrate, about which most is known. Effects of SCFA can be divided into those occurring in the lumen and those arising from their uptake and metabolism by the cells of the large bowel wall.

A. Luminal Effects of SCFA and Fermentable Carbohydrates

SCFA are the principal luminal anions in humans and other omnivores. They are relatively weak acids with pK_a values ~4.8, and raising their concentrations through fermentation lowers digesta pH. Food supply is an important variable. In rats fed a nonpurified diet ad libitum, cecal and distal colonic pH values were 6.14 and 6.87, respectively, compared with 7.40 and 7.37 in rats starved for 24 h (49). When rats were restricted to 15 and 19 g of nonpurified diet/day, cecal pH was 7.40 and 7.11, respectively, compared with 6.47 in animals fed ad libitum (148). In rats fed NSP of low fermentability (e.g., cellulose or wheat pericarp-seed coat) at levels of 50–150 g/kg diet, cecal pH is high, with values of 6.7–8.2 (51, 63, 85, 286, 307, 338, 340). Cellulose is a commonly used reference fiber, and its effects depend on dietary level. Increasing cellulose from 50 to 100 g/kg diet has no effect on pH (7.55 and 7.70, respectively) but lowers SCFA concentrations significantly from 102 to 70 mM (307). When rats are fed fermentable carbohydrates such as OS, NSP, or RS or fermentable cereal fractions such as wheat aleurone or whole flour, cecal pH is lowered by 1–2 units (60, 63, 145). Some authors (e.g., Refs. 51, 338) have reported a strong negative relationship between cecal SCFA and pH, but in other studies (e.g., Ref. 307) the relationship is absent. This may reflect the buffering by the gut contents or the presence of other dietary components, e.g., calcium which can modify pH (341). The lower limit of pH in these studies seems to be ~5.

pH values are lower in pigs fed diets that raise large bowel SCFA (20, 124, 185, 303). However, pH values change along the porcine large bowel differently to rats. In rats, proximal colonic pH may be higher than in the cecum and distal colon. In pigs, pH values are higher in the cecum, equal or lower in the proximal colon, with a continuous gradient of rising values toward the distal colon. Importantly, distal colonic or fecal pH is not necessarily predictive of conditions in the proximal bowel of pigs. In animals fed low-fiber diets (20–30 g NSP/kg), proximal colonic pH is ~7.1, rising to 7.5 in the distal colon. Values are lower when diets containing additional fiber (21, 124, 303), high RS foods (303), or high RS starches RS (185) are fed. The gradient was maintained in these trials, and one study failed to find any effect of an RS on SCFA or pH, but that may reflect the rapid fermentation in that experiment (301). Data from human interventions are limited largely to fecal values. When volunteers have consumed fermentative substrates such as lactulose (104), wheat bran (165, 170), oat bran (154), RS (214), partially hydrolyzed guar gum (289), or high-fiber diets (196), pH values are lowered significantly. Other studies with fructo-oligosaccharides (36, 312) or RS (298, 314) have failed to show such lowering. The actual pH values recorded vary by study. For example, Takahashi et al. (289) showed that control pH values were 6.17–6.25, falling to 5.4–5.5 when subjects were fed partially hydrolyzed guar gum. In contrast, Noakes et al. (214) found that mean pH was 6.18 when RS was fed and 6.40 with a low-RS diet. One of the contributory reasons for these differences may be the absolute value of SCFA as Segal et al. (265) recorded a strong ($r = -0.704$) negative relationship between fecal SCFA and pH. This means that the absolute SCFA concentrations may override other influences on pH (e.g., buffering by gut contents).

Lower pH values (and raised SCFA) are believed to prevent the overgrowth of pH-sensitive pathogenic bacteria, although this is based largely on in vitro incubation studies. For example, propionate or formate have been shown to kill *E. coli* or *Salmonella* at high (pH 5) acidity (62). Some animal studies support such findings, with greater SCFA production having been reported to lower the numbers of potential pathogens (such as *Salmonella*) in swine (231). However, the rapid weaning of piglets to diets high in fermentable carbohydrate (RS and NSP) leads to raised large bowel SCFA, colonization with a bacterium (*Serpulina hyodysenteriae*), and appearance of clinical symptoms including diarrhea (228). The syndrome seems to result from the commercial practice of very abrupt introduction of solid food rather than any adverse effect of SCFA (31). There are relatively few studies in human diarrhea, but it appears that SCFA can assist in the management of antibiotic-induced and infectious diarrhea. Fecal SCFA are lower during the active phase of cholera disease, while their elevation (through feeding of RS at 40 g/l of oral rehydration solution) diminishes fluid loss substantially and speeds remission by up to 50% (236). Diarrhea has been shown in rats when they

are fed purified diets containing very high levels (10–15%) of water-soluble polysaccharides such as gum arabic (307). This same reaction occurs in humans when the load of water-holding carbohydrate exceeds the fermentative capacity of the microflora. Normally it seems that SCFA production and absorption from RS and NSP is associated with diminished fluid loss.

Fermentable carbohydrates can alter the microbial ecology greatly by acting as substrates or supplying SCFA. Much attention has been directed toward the study of specific beneficial lactic acid bacteria, i.e., probiotics (usually bifidobacteria or lactobacilli) rather than the flora as a whole. These organisms are unlikely to change the major SCFA in the colon (31). Probiotic numbers have been enhanced by prebiotics that are defined as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and (or) activity of one or a limited number of bacterial species already resident in the colon, and, thus, improve host health" (120). Consumption of lactosucrose (218) or inulin (157) increases the fecal counts of bifidobacteria in human volunteers. Kleessen et al. (157) found that at a dose of 40 g/day, inulin consumption increased bifidobacteria numbers from 7.9 to 9.2 $\log_{10}$ cfu/g dry feces, but total bacterial counts remained unchanged. These changes were unrelated to any change in SCFA and pH. Similar bacteriological data have been reported in rats fed indigestible OS (oligofructose or xylooligosaccharides) where cecal bifidobacteria numbers were higher than in controls (51). Feeding trials in pigs with RS have shown greater fecal numbers of bifidobacteria after their oral ingestion (43). When a high amylose starch was fed, the fecal numbers were 8.91 $\log_{10}$ cfu/g wet wt compared with 8.12 $\log_{10}$ cfu/g wet wt when a low amylose starch was fed.

Some of the interaction between RS and bacteria appears to be physical, with the organisms adhering to modified or unmodified starch granules (31). This adhesion has been studied relatively little as has the interaction between RS, SCFA, and the large bowel microflora. New technologies for bacterial enumeration will facilitate development of a fuller understanding of these relationships.

B. Absorption and Metabolism of SCFA by Colonocytes

Less than 5% of bacterially derived SCFA appear in feces due to colonic uptake (195, 246, 252) which is responsible for the major decline in concentrations along the large bowel. Intubation studies have shown that SCFA are taken up from the perfused human large bowel in a concentration-dependent manner (252). In the guinea pig proximal and distal colon, this uptake was not saturable up to a concentration of 120 mM (237). At least 60% of that uptake is effected by simple diffusion of protonated SCFA involving hydration of luminal CO_2, while the remainder occurs by cellular uptake of ionized SCFA involving cotransport of Na^+ or K^+ (101). SCFA uptake is associated with a transport of water that appears to be greater in the distal than the proximal colon (38). The reduction in SCFA levels in antibiotic-associated colitis may be responsible for some of the diarrhea because SCFA stimulate colonic fluid and electrolyte transport (66), although an inhibition of NSP breakdown (with attendant laxation) is possible (204). Enhanced fluid transport helps to explain the accelerated remission from cholera seen with feeding RS. Na^+ and K^+ were thought to be the principal cations absorbed (91). However, the feeding of oligosaccharides to rats prevents osteopenia following gastrectomy (219) and increases the apparent absorption of Ca^{2+} and Mg^{2+} (338). A human study has shown greater calcium retention after consumption of fermentable carbohydrate, inulin and beet fiber (72). Apparent colonic absorption was increased significantly by inulin (33.7 vs. 21.3% in controls), but there was no change in Mg^{2+}, Zn^{2+}, or Fe^{2+} retention. Studies in humans in which SCFA have been infused into the rectum support a direct stimulation of Ca^{2+} absorption (308). A recent study in pigs has shown that apparent colonic Ca^{2+} absorption was increased by ~20% with consumption of RS (32). This increment was largely in the proximal colon, i.e., where SCFA are highest.

The major SCFA are absorbed at similar rates in various regions of the large bowel (91). Acetate, propionate, and butyrate are absorbed at comparable rates in humans (252) and rat cecum (311) and colon (101). There are regional differences in guinea pigs, with acetate clearance being high in the proximal colon and low in the cecum and distal colon (91). Under the pH conditions (5.5–7.5) thought to apply normally in the human colon, >50% of SCFA would be present in the dissociated form. However, experimentally induced changes in pH within this range affect absorption rates relatively little. This may be due to a putative unstirred layer where reassociation may occur (91), which suggests that any regional differences are due to colonocyte metabolism and not the local luminal environment. SCFA are metabolized rapidly by colonocytes and are major respiratory fuels and trophic to the small bowel and colon (331, 339). Their oxidation supplies some 60–70% of the energy needs of isolated colonocytes (244) and suppresses that of glucose (15, 244) and spares pyruvate (48). Of the three major SCFA, butyrate is the major intestinal fuel even when competing substrates such as glucose and glutamine are available (243). There is a hierarchy of oxidation, with butyrate apparently being oxidized more in the proximal than distal colon. This, coupled with lower levels and slower absorption, may be important in human distal ulcerative colitis where it has been hypothesized that

there is a defect in butyrate metabolism. Inhibition of fatty acid β-oxidation in rats through rectal infusion of 2-bromo-octanoate causes the symptoms of colitis (247). Diversion colitis occurs in human patients in those segments isolated from the fecal stream and the supply of SCFA (89). Patients with ulcerative colitis have low fecal butyrate (and pH) and high lactic acid levels during exacerbations (318). Intracolonic infusion of SCFA preparations reduce the degree of inflammation of the defunctioned segment in humans (3, 130), although this has not been confirmed (126). Butyrate enemas induced remission of ulcerative colitis (e.g., Ref. 263), but later reports have yielded inconclusive results (e.g., Ref. 278). Proliferation of cells in the upper 40% of the crypt, measured with proliferating cell nuclear antigen (PCNA), is reduced by treatment with butyrate or SCFA in patients with ulcerative colitis (261). The ratio ϕ_h is a measure of the location of the proliferative zone in the crypt and is as follows: [labeled cells in upper 40% of crypt]/[labeled cells in whole crypt]. Any increase in humans is thought to predispose to cancer risk. A lowering of ϕ_h by butyrate delivery could be of benefit, especially in the distal colon, which is the region most at risk of pathology.

C. Effects of SCFA on Colonic Blood Flow and Muscular Activity

In vitro studies have shown that incubation with SCFA (as the sodium salts) at concentrations as low as 3 mM dilate precontracted colonic resistance arterioles in isolated human colonic segments (204). Acetate and propionate were most effective. Rectal infusion of SCFA into human surgical patients leads to 1.5- to 5.0-fold greater splanchnic blood flow (203). Greater colonic blood flow has been observed with infusion of acetate, propionate, or butyrate (separately or as a mixture) into the denervated canine large bowel (162). When acetate, propionate, and butyrate were infused at 75, 30, or 30 meq/l, respectively, blood flow rose by 18.1 and 3.1% for acetate and propionate, respectively, but fell by 3.4% when butyrate was infused. The mechanism of action of SCFA on blood flow does not involve either prostaglandins or α- or β-adrenoreceptor-linked pathways (204). The presence of SCFA (as the sodium salts) in rat colon incubated in vitro leads to increased contraction that persists for ~1 min after application of SCFA solutions of up to 10 mM (337). The maximal effect was achieved at 0.1 mM with an order of effectiveness of acetate << butyrate < propionate. At higher concentrations (100 mM), contractile activity was abolished (276). The mechanisms of action may involve local neural networks as well as chemoreceptors together with direct effects on smooth muscle cells (61). SCFA produced in the colon and entering the portal circulation seem to influence the upper gut musculature. Manometric studies in humans have shown a decrease in gastric tone giving an expansion of volume after ingestion of fermentable carbohydrate (lactulose) or rectal infusion of lactose or SCFA (249). The decrease was not obviously linked to circulating peptides of intestinal origin (glucagon-like peptide 1, oxyntomodulin-like immunoreactivity, or peptide YY). SCFA appear to activate the ileocolonic brake directly in a dose-dependent manner. This effect was assayed by increases in volume in a barostat bag inserted in the volunteer's stomach with a greater rise in volume showing slower transit. The integrated changes in volume with time were 56×10^3 and 84×10^3 ml/min for 54 and 90 mmol SCFA, respectively, compared with 5×10^3 ml/min when the control solution was infused. These actions are important for the maintenance of the function of the whole gastrointestinal system, not just the colon. Slowing of upper gastrointestinal passage of food would be expected to improve nutrient digestion, whereas more rapid transit of food through the colon is thought to improve laxation. It is expected that greater blood flow enhances tissue oxygenation and transport of absorbed nutrients.

D. Trophic Effects of SCFA and the Maintenance of a Normal Colonic Cell Phenotype: Role for Butyrate and Propionate

In rats, SCFA stimulate the growth of colorectal and ileal mucosal cells when they are delivered colorectally or intraperitoneally (159, 254). Other animal studies have shown that SCFA supplementation of total parenteral nutrition (TPN) infusions retards the mucosal atrophy seen after massive bowel resection in rats (158). Feeding of diets high in fermentable carbohydrates to rats promotes ileal growth and raises ileal and cecal glucagon-like peptide-1 mRNA levels (238). In addition to promoting growth, the major SCFA (especially butyrate) appear to lower the risk of malignant transformation in the colon. In normal rats, butyrate at concentrations of 10 and 25 mM enhance proliferation only at the crypt base resulting in a fall in ϕ_h. This effect is blocked by 5 mM deoxycholic acid (316), although the cotreatment does not reverse deoxycholate-induced increases in colon weight and indices of cell proliferation (317). Secondary bile acids are cytotoxic, and in rats fed deoxycholate plus cholesterol, cell proliferation as measured by incorporation of [^{3}H]thymidine was increased (167). When the diet contained 0.15% deoxycholate plus 1% cholesterol, incorporation was 81.4 versus 43.4 dpm/μg DNA in controls. This change appears to be associated with greater susceptibility to the development of cancer (82, 173, 292). Normal mucosa from colorectal carcinoma patients resists bile-acid induced apoptosis, implying that high levels of bile acids may select for cells resistant to apoptosis (223). Some of the

effects of SCFA may be due to low intra-colorectal pH rather than any specific SCFA. At a pH of 6, bile acids are largely protonated and insoluble and so would not be taken up by colonocytes (235), and lower pH inhibits the bacterial conversion of primary to secondary bile acids (176, 213) and so would lower their carcinogenic potential.

Tumorigenesis is a multistep process with a progression from a hyperproliferative epithelium to preinvasive and metastatic carcinoma via formation of aberrant crypts and various stages of dysplasia (342). Genetic alterations are believed to occur at each step, but their determinants are uncertain and it is unclear whether butyrate opposes any or all of them in vivo. The greater proliferation with butyrate is a paradox in that it could be expected to increase risk of tumor formation. The answer may lie in the differential effects of butyrate on apoptosis in normal and tumor cell lines. In the absence of butyrate, normal colonic cells in culture undergo apoptosis within 150 min, paralleled by a fivefold increase in Bax protein gene expression (131). In contrast, butyrate leads to growth arrest, differentiation, and apoptosis in tumor cell lines (18, 81, 115, 121, 127, 128, 328). In these studies, differentiation was assessed by increased expression of the brush-border glycoproteins, alkaline phosphatase, and carcinoembryonic antigen. Normal colonic cells show decreased expression of these markers after incubation with 1–4 mM butyrate (121). SW620 cells became arrested at G_0-G_1 and G_2-M within 12 h of exposure to butyrate with apoptosis 4 h later that was related to abnormalities in the mitochondrial electron chain (133). Siavoshian et al. (268) demonstrated changes in the cell cycling factors p21 and D in HT-29 human colonic adenocarcinoma cells. Heerdt et al. (132) demonstrated elevated alkaline phosphatase in cancer cells treated with butyrate, particularly in the floating apoptotic cells, suggesting that programmed cell death occurred subsequent to differentiation. One of the possible mechanisms for differentiation in tumor cells in vitro is a reduction in nuclear levels of the protooncogene c-*myc* (68, 291), a factor which is important in control of tumor growth. Treated cells have a significant loss in colony-forming capacity in soft agar (156) which is related to the lowering of c-*myc* (291). Butyrate treatment also reduces cytoskeleton-associated tyrosine protein kinase activity (264), which is important in cellular responses to cytokines such as transforming growth factor-β_1 that promote growth in HT-29 tumor cells (24). Inhibition of DNA synthesis may occur through inhibition of histone deacylase (160) as removal of histones is an important first step in DNA replication. Apoptosis may be pivotal in the progression from colon adenomas to colon carcinomas as mutations in genes, such as p53, which control programmed cell death are often seen in tumors (251, 256). Apoptosis is enhanced by butyrate but not through the p53 gene (128, 132). Propionate and acetate also induce apoptosis but to a lesser extent than butyrate (127) and at higher concentrations (≥40 mM), although these can be achieved in colonic digesta in vivo. This accords with the differential effects of the three fatty acids in inhibiting proliferation and inducing differentiation (115, 328). Additionally, propionate induces apoptosis in adherent cells, but butyrate induces it only in floating cells (127, 128, 132), suggesting differential metabolic effects of the two SCFA. Cells may lose their responsiveness to butyrate (30, 329), with some cancer cell lines resisting butyrate-induced apoptosis (127). Butyrate and acetate (but not isobutyrate or propionate) also appear to inhibit DNA oxidative damage due to H_2O_2 in isolated rat distal colon cells at concentrations of 6.25 M (1). Surprisingly, a mixture of the major SCFA did not oppose the genotoxicity.

It has been shown in rats treated with a carcinogen (AOM) that apoptosis was increased in aberrant crypt foci when large bowel butyrate was increased through feeding it in slow release pellets (50). Cecal and colonic butyrate concentrations were ~85 and 9 mM after the treatment, and apoptosis (measured by TUNEL) was increased from 0.12 in controls to 0.81. Apoptosis measured morphologically was rather higher in controls and lower in the butyrate-treated group but was still significantly different. Neither cell proliferation nor aberrant crypt foci induction were changed. One study has shown oral butyrate stimulates tumor promotion in rats treated with dimethylhydrazine (111). Plasma butyrate levels were not measured, so it is unknown if the transformed cells were exposed to a higher butyrate concentration. However, if oral butyrate were absorbed like propionate (as appears likely), then most would be absorbed via the upper gut and cleared by the liver (147).

Inter alia, the experimental data support a role for SCFA in promoting a normal phenotype in colonocytes that is beyond the provision of metabolic substrate. It appears that butyrate and (to a lesser extent) propionate act to prevent the development of abnormal cell populations. A direct role for these SCFA in the prevention of human colorectal carcinoma remains to be established.

V. NUTRITION AND LARGE BOWEL SMALL-CHAIN FATTY ACIDS

The influence of weaning and gender (as well as heredity) on SCFA have yet to be explored in detail. Fecal data suggest that gender may be an important factor for NSP and RS. Lampe et al. (165) have shown that the digestibility of dietary fiber (measured as NDF) in wheat bran was 43% in women and 37% in men when they consumed 30 g of wheat bran fiber/day. Fecal bulking was lower and mouth to anus transit was longer in women than in men and varied with fiber type (vegetable vs.

wheat) and level of intake (10 or 30 g/day). In women consuming a low-fiber diet, the loss of starch into the colon (as measured by breath H_2) after a standard test meal is 30% lower during the luteal phase of the menstrual cycle than during the follicular phase (188). The respective calculated mean values were 9.7 and 6.6 g/50 g starch, and stool weight was also lower during the former phase. These differences warrant further investigation.

A. Fermentable Carbohydrate Supply and SCFA

Of the techniques available and applied widely, only in vitro fermentation gives an estimate of SCFA production. Breath gas evolution is an indicator of fermentation while measurements in human or animal feces or digesta measure increases or decreases in concentrations or pools. These are taken to reflect altered production, which appears to be reasonable (but imprecise) when ones considers studies in cannulated animals that show greater portal venous SCFA concentrations and transport after feeding of fermentable carbohydrates (123, 147, 239).

1. Total SCFA

A) IN VITRO STUDIES. Of the methods used widely, only in vitro incubation offers a means of assessing SCFA production. Animal and human studies generally report changes in concentration or pools and take these to be an index of formation. Increased SCFA production by human fecal inocula has been demonstrated with fiber-rich foods including bran fractions from wheat, oats, barley, corn and rice, soybean fiber, vegetable extracts, and pea fiber (25, 73, 189, 207). Purified preparations (such as glucose, cellulose, guar gum, pectin, and starch) and isolates (e.g., from vegetables) also have been examined (25, 73, 189, 207, 324). Some purified carbohydrates (such as cellulose) are fermented slowly and incompletely while glucose is fermented quickly and completely. This quality is referred to generally as fermentability, a term which combines the rate and extent of carbohydrate degradation. It is highly variable with ~97% of pectin and only 6–7% of cellulose and maize bran being fermentable (25, 37). Less than 50% of wheat bran components are fermented, whereas estimates for psyllium fall in the range 20–50% (285) and ~96% of oat bran is lost (58). High fermentability relates to greater SCFA production in vitro. Fermentation of 30 mg glucose, pectin, or cellulose/ml yielded concentrations of 220, 172, and 23 mmol total SCFA/l, respectively, in the incubation fluid (203). Additionally, greater fermentability may be associated with a more rapid fermentation (25). The large European interlaboratory study that examined a number of fiber sources showed a close relationship between NSP degradation and SCFA production (25). Similar relationships between RS degradation and SCFA production have been noted with pig fecal inocula (185). The European fiber study was of sufficient size to allow for the variables that can impact on fermentation and showed clearly the wide range of values obtained in five laboratories (Table 4). A similar range could be expected for RS, and appropriate steps can be taken to minimize it.

B) ANIMAL STUDIES. Cecal and colonic SCFA fall in starved rats or rats fed restricted amounts of a nonpurified diet (49, 148), with the greater fall occurring in the colon (49) (Table 5). These changes were reversed by restoration of unrestricted feeding, although the distal colonic values were slowest to recover. There have been numerous studies in rats with measures generally confined to the cecum. These experiments confirm that enrichment of the diet with NSP, RS, and OS leads to elevation of large bowel SCFA. Feeding diets containing complex fiber mixtures (187) or fiber-enriched cereal fractions such as wheat bran (60, 107, 191, 193, 248), oat bran (146, 344), barley bran (191), wheat aleurone (60), or rice bran (107) leads to higher concentrations of SCFA in large bowel digesta. For example, concentrations of 50–70 mM have been recorded in rats fed diets low in fermentable carbohydrate (cellulose or wheat pericarp-seed coat) with values as high as 180 M in rats fed fermentable carbohydrate (wheat aleurone) at levels of 100 g/kg diet (60). Similar increases have been shown with polysaccharide isolates including pectin (143, 346), inulin (248), guar gum (86), gum karaya (86), xanthan gum (86), and gum arabic (286, 305, 307). The feeding of OS (51, 219) and RS (125, 187, 341) also increases cecal SCFA. In many of these reports, the weight of large bowel digesta was increased leading to cecal SCFA pools, which were 100–300% higher than in controls depending on carbohydrate source and dietary inclusion level. Assuming that absorption rates were not changed greatly, pool size gives a rough measure of SCFA production rates that indicate substantial enhancement by RS, OS, and fiber.

TABLE 4. *In vivo and in vitro substrate fermentability and production of SCFA*

Fiber Source	Incubation Time, h	Fiber Degradation, %	SCFA Produced, mM
Pectin	10	89 (69 to 112)	62 (45 to 83)
	24	97 (83 to 117)	68 (46 to 97)
Sugarbeet fiber	10	32 (1 to 70)	26 (6 to 54)
	24	60 (14 to 98)	45 (29 to 72)
Soybean fiber	10	72 (55 to 95)	51 (29 to 70)
	24	91 (80 to 98)	64 (41 to 79)
Maize bran	10	0 (−28 to 21)	5 (−2 to 11)
	24	6 (−21 to 27)	12 (3 to 26)
Cellulose	10	9 (−9 to 38)	2 (−1 to 4)
	24	7 (−15 to 45)	3 (0 to 7)

Values in parentheses indicate range. [Modified from Barry et al. (25).]

TABLE 5. *Changes in large bowel SCFA in rats with starvation and refeeding with a nonpurified diet*

		SCFA, mM			
	Region	Acetate	Propionate	Butyrate	Total
Time of starvation, h					
0	Cecum	55	27	33	122
	Proximal colon	20	6	6	32
	Distal colon	43	13	15	73
56	Cecum	47	10	5	59
	Proximal colon	0	0	0	0
	Distal colon	4	0	0	5
Time of refeeding, h					
5	Cecum	38	17	11	63
	Proximal colon	27	10	6	42
	Distal colon	18	6	3	21
15	Cecum	72	23	14	106
	Proximal colon	58	16	11	89
	Distal colon	39	12	4	56

[Modified from Butler et al. (49).]

When intakes of dietary water-soluble carbohydrates exceed the fermentative capacity of the microflora, SCFA fall due to osmotic diarrhea secondary to their presence in the digesta.

In pigs, feeding fiber or RS elevates large bowel SCFA concentrations and pools. When pigs were fed a western-type diet (i.e., high in fat and protein and low in fiber), increasing fiber intake from ~14 to 42 g NSP/day by feeding wheat bran raised total digesta from 255 to 498 g and SCFA pools from to 12.7 to 32.2 mmol (303). However, NSP were not predictive of changes in digesta mass and SCFA, so when pigs were fed ~40 g NSP as navy beans, these were 655 g and 60 mmol, respectively. The disparity appears to be due to the RS present in these foods, which can add considerably to their fermentable carbohydrate content. Fleming et al. (102), using cannulated pigs, noted a similar expansion when beans were fed. Broadly similar data have been obtained with white rice plus rice bran and brown rice with the latter resulting in a disproportionate rise in digesta and SCFA (32, 184).

c) HUMAN STUDIES. Controlled dietary studies in humans are few and generally limited to fecal measurements. One such trial showed that consumption of an additional 10–30 g of fermentable carbohydrate/day (as wheat bran or vegetable fiber) raised fecal SCFA (110). However, these supplements also raised fecal bulk and shortened transit time (164, 165), which could have raised fecal SCFA without any change in production. Other studies in which there was no change in laxation indicate that greater intake of fermentable carbohydrate results in higher SCFA production (e.g., Refs. 214, 314). Fecal SCFA concentrations and excretion have been shown to be higher with feeding of some NSP such as partially hydrolyzed guar gum (289) but not others (oat bran) (214). Various sources of RS (74, 153, 214, 225, 267, 314) and acarbose (142, 259, 326) raise fecal SCFA. These increases have been reported as higher concentrations, excretion, or both, which may reflect passage of the fecal stream. This is likely to be the reason for the lack of effect of OS and RS at low doses on fecal SCFA as they would be expected to be fermented relatively rapidly, and the products of fermentation could be absorbed in the more proximal colon.

2. *Individual SCFA*

a) IN VITRO STUDIES. Acetate is the most abundant SCFA in fecal and digesta samples and formed in vitro. Fecal inocula from adults and children produce lesser amounts of propionate and butyrate, whereas inocula from breast-fed infants produce little or no butyrate. In the latter, acetate, ethanol, propionate, and formate are the main products (333). Rodent studies suggest that propionate and butyrate production are related inversely. Fiber sources (such as oat bran) that lower plasma cholesterol through enhancing steroid excretion raise the contribution of propionate relative to butyrate, whereas wheat bran has the opposite actions (59). The relationship seems to be secondary to changes in large bowel steroids because feeding of diets containing cholesterol plus cholic acid to rats lowers cecal butyrate to <1 mM (96). Moreover, in rats fed wheat fractions, there is a negative correlation between cecal butyrate and steroids (total and individual bile acids and neutral sterols) and a positive correlation between the latter and propionate (145). The correlations were strong between deoxycholate and butyrate ($r = -0.66$, $P < 0.001$) and propionate ($r = +0.55$, $P < 0.01$) (Fig. 2). Whether such relationships occur in humans is not established.

The in vitro data indicate that substrate influences propionate and butyrate production considerably. Barry et al. (25) showed that 24-h production of total SCFA and

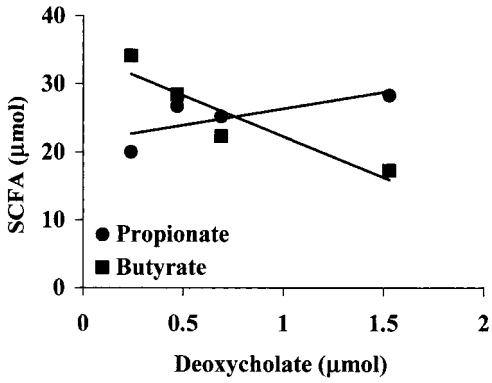

FIG. 2. Relationship between the pools of short-chain fatty acids (SCFA) and deoxycholic acid in the cecal contents of rats. Rats were fed purified diets containing milled whole wheat or wheat bran, wheat pollard (a bran-rich flour), or white wheat flour obtained from that flour. SCFA and bile acids and neutral sterols were measured in cecal contents at the end of the feeding trial. Each data point represents the mean values for each of the four dietary groups and shows that there were strong negative correlations between the total pool of deoxycholate (a secondary bile acid) and the pool of butyrate. Conversely, there was a strong positive relationship between deoxycholate and the pool of propionate. [Modified from Illman et al. (145).]

butyrate was high with pectin, intermediate with soybean fiber, and low with sugarbeet fiber (Table 4). However, this study and others (e.g., Ref. 203) have shown strong time and concentration dependencies that limit data interpretation. The latter may be particularly important in relation to human diets. For example, at 2.5 mg pectin/ml, acetate and butyrate contributed 81 and 9% of total SCFA, respectively, but at 30 mg/ml, the corresponding values were 74 and 20%.

B) ANIMAL STUDIES. Starvation or restricted feeding of rats leads to a fall in acetate that is reversed on restoration of ad libitum feeding (Table 5). Conversely, when SCFA have been elevated through feeding of foods high in NSP, OS, and RS or isolates of those carbohydrates, concentrations and (where measured) pools have been increased. Propionate and butyrate fall after starvation or restricted feeding of rats, with butyrate falling most and being restored more slowly on refeeding, especially in the distal colon (Table 5). This difference could be due either to its apparent preferential utilization by colonocytes or the time dependence observed in vitro. Fermentable carbohydrates, including fiber-rich fractions, purified NSP, OS, and RS raise the concentrations and pools of propionate and butyrate in rats. However, it is hard to extrapolate these data to humans and also to link the fermentation of individual carbohydrates with particular SCFA profiles. Analysis of NSP monosaccharides suggests that the uronic acid content correlates strongly with production of acetate, while xylose content is weakly related to butyric acid production (255). Dietary NSP mixtures are more fermentable and produce quite different SCFA profiles (with higher butyrate levels) than when the NSP are fed alone (305). For example, total cecal SCFA were 171 and 157 mM and butyrate was 22 and 32 mM in rats fed either cellulose or gum arabic at 140 g/kg diet. When these NSP were fed as a 50/50 mixture, total SCFA and butyrate were 237 and 53 mM, respectively. Extrapolation of these data from rats to other model species and to humans eating mixed diets needs caution. When fiber-rich foods (wheat bran and oat bran) were fed to pigs, large bowel propionate and butyrate were similar (303) despite reported differences in rats. Portal venous butyrate concentrations were higher in pigs fed oat bran than in those fed wheat bran (304), and Bach Knudsen et al. (21) showed in pigs that oat β-glucan raised large bowel butyrate. In this species, low-fiber diets depress butyrate and propionate in pigs, especially in the distal colon (184, 303). When pigs were fed physiological RS as either beans or brown rice, the distribution of SCFA was quite different. Butyrate was raised more with brown rice (especially in the distal colon), whereas with beans the increase was greater for propionate in the proximal bowel. Martin et al. (185) have noted similar distributional differences between RS types.

C) HUMAN STUDIES. The limited human data are contradictory. Greater fecal excretion of butyrate and propionate has been observed with consumption of wheat bran compared with vegetable fiber (110). In contrast, feeding of partially hydrolyzed guar gum resulted in greater fecal excretion of all three major acids, but no change in the concentration of propionate and butyrate and a decline in their relative contribution (289). The data for RS are more consistent, and several studies have shown that its consumption raises fecal butyrate (214, 225, 314). Given the putative importance of butyrate in maintaining large bowel function, these (and other experimental and epidemiological findings) have focused attention on RS as a fermentative substrate for the colonic microflora.

VI. SMALL INTESTINAL POLYSACCHARIDE DIGESTION AND LARGE BOWEL CARBOHYDRATE SUPPLY

NSP and non-starch-derived oligosaccharides are virtually completely resistant to digestion by the intrinsic enzymes of the human stomach and small intestine. The reported recovery of ingested fiber components in ileal effluent of ileostomates consuming whole foods was 97% (151). The excretion of inulin and oligofructose in ileostomates was close to 100% (11), whereas that of uronic acids (alginate) was 95% (257). The (1→3),(1→4)-β-D-glucans found principally in oat and barley grains at concentrations of 3–12 g/100 g total seed wt appear to be exceptional. At low intakes (1.8 g β-glucan/day), 80% was degraded, and at higher intakes (16 g/day), 30% was lost on transit from the mouth through the small intestine of human ileostomates (8, 9). Similar data have been ob-

tained with pigs cannulated in the terminal ileum (20). Twenty-five to 36% of oat β-glucans was recovered at this site compared with 82–104% of wheat NSP. β-Glucans could be limited substrates for human and porcine α-amylase. However, ileostomy effluent contains measurable quantities of SCFA (200), so the degradation in humans and pigs may be bacterial. This is supported by the fact that the last few centimeters of the ileum appear to take on the appearance of the cecum in patients with stable ileostomy (140). Normally, ileal NSP digestibility is virtually zero.

A. Starch Digestion

Small intestinal starch hydrolysis is effected first by α-amylases that release maltodextrins that are then hydrolyzed by membrane-associated maltases to free glucose which is absorbed. RS is determined by the factors that control this small intestinal digestion. These include starch structure, the presence of other food components (NSP, lipids), cooking, industrial processing, and individual physiological influences such as chewing and transit (13).

1. Starch structure and ileal digestibility

NSP are an extremely diverse group of homo- and heteropolymers. In contrast, starch is a glucose homopolymer found in two forms: amylose and amylopectin. The former is an essentially linear structure where the glucose units are joined by α(1→4) glycosidic links while amylopectin consists of linear α(1→4) linked chains with α(1→6) linked branch points. Amylose has degrees of polymerization (DP) of 100–10,000 monomer units, whereas the molecular weight of amylopectin can exceed 10^7 (for general reviews, see Refs. 13, 22, 31, 114). In raw foods, starch is present as crystalline granules with two main forms (A and B) that differ in the relative proportions of amylose and amylopectin. A-form starches have chain lengths of 23–29 glucose units and are found in cereals. Tuber and amylose-rich starches are B form with 30–44 glucose units. Both have similar molecular arrangements with left-hand parallel double helices, but the B form has more associated water. A third form (C) is found in legumes and appears to be a mixture of A and B forms that resists digestion as do B-form starches. The positions of the branch points relative to the linear regions of amylopectin dictate its cluster and structural properties in the crystalline region of the starch granule. The majority of the amylose exists in the amorphous regions of the starch granules, while the amylopectin gives starch its crystalline structure. When raw starches are heated with limited water, they "melt," which leads to loss of order in the crystalline region and can take up the V form in which starch becomes associated with lipid. These V-form complexes appear to be digested incompletely in the small intestine of dogs (212). When starches are cooked with water, the granules become gelatinized to a degree that depends on temperature, proportion of water, and time of cooking, but above 90°C much of the crystallite order is lost through breaking of the intermolecular forces allowing penetration of water (69). Gelatinized starches are digested much more rapidly than raw ones. Starch gels are unstable, and B-type crystallites form on standing at cool temperatures. This process is termed retrogradation and results from the reassociation of the linear regions of the polymers to form insoluble crystallites containing short linear segments of α-(1→4)-linked glucose units (122) that resist enzymatic hydrolysis (269). Retrogradation occurs when foods are cooked and then cooled. Generally, there is a negative relationship between the amylose content of a starch and the onset and peak minimum temperature of gelatinization (109). High amylose starches (such as those from various maize cultivars) require higher temperatures and pressures fully than high amylopectin starches to be gelatinized (69). Thus high amylose starches are intrinsically more resistant than those higher in amylopectin and retrograde more readily. Starch processing is important as repeated autoclaving and cooling increases the amount of resistant starch and slows starch digestion overall (108).

B. Classification of RS

The name *resistant starch* was coined to describe the incomplete digestion in vitro of starch in foods that had been cooked and then cooled (29) but now includes all starch and starch degradation products that resist small intestinal digestion and enter the large bowel in normal humans (16). It is defined strictly in terms that exclude the small intestine. Small intestinal amylolysis can occur at different rates, but only incompletely digested starch can contribute to RS (310). RS was originally classified into three main types (94), but a fourth has been added (42) (Table 6). RS_1 includes that trapped within whole plant cells and food matrices (e.g., partly milled grains and seeds) where there is a physical barrier

TABLE 6. *Nutritional classification of resistant starches*

Types of Resistant Starch	Examples of Occurrence
RS_1; physically inaccessible	Partly milled grains and seeds
RS_2; resistant granules	Raw potato, green banana, some legumes, and high-amylose starches
RS_3; retrograded	Cooked and cooled potato, bread, and cornflakes
RS_4; chemically modified	Etherized, esterified, or cross-bonded starches (used in processed foods)

[As classified by Brown et al. (42) and Englyst et al. (94).]

to amylolysis. The presence of intact cell walls contributes to the RS content of legumes. More extensive milling (and chewing) can make these starches more accessible and less resistant. RS_2 comprises those granules from certain plants that are gelatinized poorly and hydrolyzed slowly by α-amylases (e.g., raw potato and green banana, high amylose maize starch). RS_3 comprises retrograded starches, and examples are cooked and cooled rice or potato. RS_4 comprises the chemically modified starches (e.g., ethers or esters) that are used by food manufacturers to improve the functional characteristics of the starch. Although these modified starches are found very widely in processed foods, neither their contribution to RS intakes nor their physiological effects have been studied widely. Studies in vitro with purified pancreatic amylases have shown little effect of chemical modification on rate of hydrolysis of tapioca starch but a lower degree of hydrolysis of waxy maize corn distarch adipate (168). Several long-term studies (80, 141, 297) suggested that modified starches could function as RS as judged by increased cecal digesta masses, consistent with escape of carbohydrate into the colon. In vitro incubations have shown that modified starches resist amylolysis in proportion to the degree of substitution (336). More recent studies by Raben et al. (234) have examined the blood glucose responses in humans after consumption of starches modified by β-cyclodextrinisation or by acetylation. They found lower values with the latter compared with control starches, suggesting diminished small intestinal hydrolysis. A preliminary report has shown that acylated starches function as RS and raise large bowel SCFA in rats through release of the esterified acid and fermentation of the starch (13). However, the type of modification may be important because Ebihara et al. (85) reported that hydroxypropylated starch raised cecal digesta but not SCFA. Evidently, determining the effects of the different types of RS (including RS_4) in whole foods and mixed diets is important.

C. Determination of RS in Foods

RS has been measured chemically as that starch not hydrolyzed after 2 h of incubation at 37°C with pancreatin (containing α-amylase plus proteolytic and lipolytic enzymes), amyloglucosidase, and invertase (94). These conditions approximate those in the human small intestine. The assay has been validated in extensive ileostomy studies, although there is individual variation of 74–126% of RS starch fed, depending on the food source (95, 271). Berry (29) and Muir and O'Dea (210) used incubation at 37°C for 15–16 h, with the latter providing in vivo validation (through parallel measurement in ileostomists). As yet, there is no physiologically relevant measure of RS for foods as they are eaten, and any analysis needs to allow for the presence of other inhibitory food components (amylase inhibitors, lipids, NSP, etc.) as well as physiological variables including chewing and individual variation in transit (13, 55, 271). Sample pretreatment through milling, grinding, or chewing can all influence the final outcome. One way to encompass these factors is to use standardized mincing (94). When this is done, the results correlate highly with the amount of starch recovered in ileostomy effluent (which is one of the few means of assessing starch digestion in humans in vivo). However, the method is labor intensive and gives poor reproducibility unless conducted by highly trained personnel and so has not been adopted widely outside Britain. Muir and co-workers (209, 210) have developed a more physiological procedure that mimics chewing and gastric digestion. Their in vitro data correlate reasonably well with the starch recovered in ileostomy effluent. The range of foods they have examined was quite small, the amount of resistant starch fed was low, and the number of volunteers used was restricted. Åkerberg et al. (5) have modified the Muir procedure (to standardize the chewing step) and also extended the range of foods examined and obtained good agreement ($r = +0.97$) between values measured in foods in vitro and in vivo. Clearly, such a suitable methodology to measure RS needs to be applied widely so that the RS content of foods and the RS intakes of individuals and groups can be estimated with confidence.

VII. RESISTANT STARCH IN THE LARGE BOWEL: COMPARISONS WITH NONSTARCH POLYSACCHARIDES

A. Relative Contributions of RS and NSP to Large Bowel Carbohydrate Supply

1. Evidence for incomplete starch digestion in humans and estimates of RS intake

The "carbohydrate gap" is the discrepancy between NSP intakes and calculations of bacterial activity of the large bowel microflora (281) and supports a significant contribution by RS. Individuals in affluent westernized countries may consume up to 28 g NSP/day (22). However, much larger quantities, possibly as much as 80 g, of fermentable carbohydrate are needed to sustain the biomass and account for SCFA production (281), and NSP may only provide 25% of that requirement (139). With the use of portal venous SCFA data from patients and estimates of human portal venous blood flow, production rates of 163 mmol/day (fasting) and 353 mmol/day (postmeal) have been estimated (73). Livesey and Elia (175) calculated a maximal yield of 0.6 g SCFA/g carbohydrate fermented. Using this value, Cummings (73) estimated that ~32–42 g of carbohydrate would need to be fer-

mented daily to produce 300–400 mmol of SCFA, which would contribute ~2–4% of daily energy. More direct measures are possible in animals. Using an electromagnetic meter to measure portal venous blood flow, SCFA were shown to provide ~30% of the total energy requirements of a 62-kg pig fed twice daily (239). When pigs were fed single meals sufficient to provide 50% of their daily energy needs, net SCFA absorption was 800–1,429 mmol/day (123, 239). This translates to >120 g carbohydrate/day, which is higher than those estimated in humans. In pigs fed a diet containing 6% cellulose as the fiber source, the quantity of SCFA transported (1.18 mol) was considerably greater than that which could be supplied by fermentation of the 48 g of fiber in the diet.

In humans, RS and OS could close the carbohydrate gap (274), but consumption of OS appears to be self-limiting due to osmotic effects and may contribute only 5–10 g/day. Direct evidence that a physiologically significant amount of starch reached the terminal ileum (and could enter the colon) was shown in intubated volunteers (283). Substantial quantities of starch (and other macronutrients) were found in ileal effluent after consumption of certain foods such as beans and high amylose starch. Thus, in a highly digestible food such as white bread, only 2.8% of available carbohydrate (i.e., starch) appeared in the effluent compared with 13.8% with lentils and 22.6% with high amylose bread (279). The fiber content of the food was found to be an important determinant of digestibility, and greater fiber content also increased ileal protein losses. Muir et al. (209) compared high and low RS meals and showed that of meals containing ~52 g of starch, ~4% (1 g) was undigested with low RS food and 48% (25 g) with high RS food. Silvester et al. (271) showed that RS intakes varied from 0.4 to 34.8 g/day, depending on dietary starch content and type. Each type of RS classification has discrete influences on the quantity entering the large bowel. For RS_1, which is starch physically entrapped within the food, the degree of milling is an important factor (172, 210). The comparison made by Muir et al. (209) was of a mixture of bread cooked with high-amylose starch, uncooked green banana flour, and coarsely ground uncooked wheat (high RS) versus cooked, low-amylose starches (low RS). $RS_{1–3}$ are subject to a range of influences that could change their levels in processed foods. Cooking conditions are very important in determining the amounts of RS_2 and RS_3 through gelatinization and retrogradation. Chewing also decreases the amount of RS present by reducing particle size (210). Rapid small intestinal transit time is likely to deliver more starch into the large bowel (271), and smaller particles have slower rates of small intestinal passage than do large ones (137). The importance of this variable has been confirmed in cannulated pigs in which starch concentrations were equally higher when they were fed coarse brown or white rice as opposed to fine brown or white rice (32, 184). Even though high levels of NSP (as occurs in brown rice) appear to inhibit small intestinal amylolysis (279), the pig data suggest that particle size is as important. Modified starches (RS_4) appear to survive the conditions that affect the other forms of RS (336), but this is yet to be established in vivo.

Although the absolute quantity of dietary NSP entering the normal human cecum and colon via the ileocecal valve can be calculated from intakes, this is not possible for RS. The RS content of most foods has not been determined, and precise values of intake for individuals consuming mixed diets are not available. Brighenti et al. (40) have made one such estimate and calculated that Italian diets provided only ~8.5 g RS/day, which appears to be a low value relative to other estimates. Overall, it seems that as more starch is eaten, more enters the colon (57, 283), and it is thought that ~10% of total dietary starch may escape digestion in the human small intestine (10, 92, 105, 106, 279, 283). Clearly, the lack of any quantitative estimate of RS limits comparison between it and NSP.

B. RS and Fecal Bulking

Dietary fiber accelerates transit, promotes laxation (171), relieves constipation (23), and protects against diverticular disease (7). This is achieved through fecal bulking, which is also associated with diminished risk of colorectal carcinoma. Cummings et al. (75) reported that death rates from colonic cancer ranged from ~20/100,000 (age standardized) in Scotland to 0.4/100,000 in Uganda. Corresponding daily fecal weights were 72–93 g and 470 g. Wheat bran is a most effective fecal bulking agent and raises stool mass by up to 4.9 g/g consumed. Less than 50% of wheat bran is fermented (216), and the passage of fermented plus unfermented material may account for its effectiveness (282). RS is of generally high fermentability, so its fecal bulking action is rather variable and much less than that of wheat bran. Increased stool weight and/or altered bowel habit have been observed in some human studies with starch (e.g., Refs. 74, 153, 267, 225, 314) or acarbose (142, 259, 326). Data from human interventions in which effects of RS on fecal bulking, pH, and SCFA have been investigated are consolidated in Table 7. Holt et al. (142) reported that over 1 yr fecal wet weight was increased from 155 to 221 g/day in individuals consuming acarbose. Other studies have failed to show any effect (106, 298). In part, these inconsistencies may reflect the relatively low incremental effect of RS on fecal bulk with 1–1.7 g additional stool/g RS consumed (74, 214, 225, 314). The variability may reflect either the effect of dosage or differences between RS types, while RS fermentation may spare that of NSP (225), which could also contribute to some of the variability through laxation. Cummings et al.

TABLE 7. *Changes in fecal variables in human interventions with acarbose or RS*

Authors	Study Design	RS Type	Diets	Fecal Mass, g	pH	Fecal Total SCFA	Fecal Butyrate
						(mmol/g wet wt)	
Scheppach and co-workers (259, 260)	Double-blind crossover (n = 12 for mass, +11 for SCFA)	Acarbose	Placebo	124[a]	NR	57.6	12.4[a]
			Acarbose	208[a]		65.8	19.6[a]
						(mmol/g dry wt)	
Holt et al. (142)	Placebo controlled, 1 yr (n = 24)	Acarbose	Placebo	155[a]	7.0[a]	452	76
			Acarbose	221[a]	6.3[a]	435	76
						(mM)	
van Munster et al. (314)	Sequential (n = 11)	RS_2	Control	119[a]	6.6	87.8	9.1
			RS_2	147[a]	6.7	89.6	10.4
Noakes et al. (214)	Crossover (n = 23)	RS_2	High-amylose starch	108	6.18[a]	119.2*	31.1[ab]
			Oat bran	100	6.22[b]	101.1	23.5[a]
Low-amylose starch				109	6.40[ab]	100.6	20.1[a]
						(mmol/kg wet wt)	
Cummings et al. (74)	Randomized crossover (n = 12, 5–10 individuals/treatment)	RS_2	R + SDS†	110	NR	98.9[a]	15.0[a]
		RS_3	Bran	201		77.1[abc]	15.8[b]
			Potato RS_2	151		99.7[b]	18.4[abc]
			Banana RS_2	161		97.5[c]	15.2[c]
			Wheat RS_3	153		83.4	17.0
			Maize RS_3	161		85.7	14.6
						(mM)	
Phillips et al. (225)	Randomized crossover (n = 11)	RS_2	Low RS	138[a]	6.3[a]	79.0[a]	19.0[a]
			High RS	197[a]	6.9[a]	99.5[a]	26.2[a]
Jenkins et al. (153)	Randomized crossover (n = 24)	RS_2 and RS_3	Low fiber	163[a]	NR	102.8	19.2[a]
			Wheat bran	258[ab]		107.9	21.3[a]
			RS	185[b]		108.1	22.7[b]

NR, not recorded. * As sum of acetate, propionate, and butyrate reported by authors. † R + SDS = rapidly + slowly digestible starch. [abc] Values sharing a common superscript are significantly different ($P < 0.05$, minimum).

(74) reported that raw potato and green banana (both RS_2) increased stool wet weight by 1.6 and 1.7 g/g, respectively. The effects of RS appear to be highly dependent on source, with green banana RS actually increasing transit time. RS_3 fed as wheat or high-amylose maize starch gave increments of 2.4 and 2.7 g/g, respectively. These effects were much less than for NSP, which increased both wet and dry matter while RS increased dry matter only. Overall, RS appears to rank alongside other highly fermentable carbohydrates such as legume NSP and pectin (73), fructo-oligosaccharides (119), polydextrose (2), or inulin (119) in its effectiveness.

C. Fermentation and Colonic and Fecal SCFA

There are two important issues in relation to RS and SCFA production: the absolute contribution and the acids that are formed.

1. In vitro studies

Some studies show that starch favors butyrate production (64, 271, 324, 326). However, individuals differ with inocula from some consistently producing more propionate than butyrate (323) and from others being incapable of metabolizing some types of RS (74). Martin et al. (185) examined the fermentation of various types of RS with pig inocula and found complete fermentation of wheat and maize starch and nearly complete fermentation of pea and potato starch within 24 h. However, only 87 and 57%, respectively, of an RS_2 and the same starch after retrogradation (RS_3) were fermented. Significantly more SCFA were recovered from maize starch (340 mM) than from wheat starch (297 mM) despite complete fermentation. Substantially more butyrate was formed from potato starch (25% of total SCFA) and the RS_2 (23%) than from the RS_3 (14%) and maize starch (14%). All of these data show that a blanket assumption that fermentation of starches is complete and yields more butyrate is unjustified. The data may need to be revisited because some of the incubations were with starch alone. Feeding trials (especially with humans and pigs) are conducted with RS plus NSP, and it is known that SCFA profile obtained with mixtures differs from that with individual carbohydrates. The role of altered bile acid excretion also needs to be determined.

2. Human breath gas measurements

There have been relatively few reports of RS and breath gas evolution, and they are equivocal and tend to dissociate breath gases from other colonic events. Achour et al. (2) showed a rise in breath H_2 that correlated with an increase in plasma acetate in humans consuming RS. Conversely, Flourié et al. (106) found that the amount of breath H_2 produced was not related to the amount of starch infused into the cecum in human volunteers. Tomlin and Read (298) fed humans with corn flakes containing 3% RS_3 and found a substantial increase in breath H_2 evolution but no change in fecal variables. Van Munster et al. (313) showed that when healthy volunteers consumed 28 g RS_2, 24-h integrated production of breath H_2 was increased relative to a control. The increment was greater in methane nonproducers, but methane evolution was increased also in methane producers. Poppitt et al. (230) examined the relationship between the amount of resistant starch ingested and the amount of hydrogen and methane excreted. Healthy men were fed a diet containing either 16 or 38 g NSP together with 16 or 19 g RS. H_2 production increased nonsignificantly, but CH_4 decreased. As noted, South African blacks consuming a high maize corn diet (likely to be a mixture of RS_2 and RS_3) had substantially higher breath gas evolution than whites with a higher fiber intake (220). However, studies with a similar mixture in a population of North American volunteers showed no change in breath gases (153). This may reflect the quantity of RS consumed, indicating that dose responses need to be established, preferably coupled with measures of SCFA production. However, this is not the only reason because high-amylose starches (known to raise colonic SCFA in animals and fecal SCFA in humans) did not raise breath H_2 in volunteers (221). These discrepancies underscore the unsatisfactory nature of breath gas tests in estimating fermentation and show that the absence of greater gas evolution after RS consumption does not indicate unchanged SCFA production.

3. Human fecal measurements

With one exception (298), RS consumption increased fecal SCFA and butyrate concentration and/or excretion. For example, Scheppach et al. (260) showed an increase in both butyrate concentration (Table 7) and excretion with consumption of acarbose. In studies with RS_2, van Munster et al. (314) showed only the former, and Noakes et al. (214) the latter. Again, the disparity may reflect either the dose or type of RS and also differences in transit (which could influence absorption within the colon). Time of adaptation may also be important as Holt et al. (142) showed in an extended feeding trial with acarbose. Quite different values for fecal SCFA were obtained at 6 and 12 mo. Furthermore, it is not clear whether the presentation of fecal values as concentrations or excretion rates is more appropriate. In a study where several forms of RS were consumed by volunteers, fecal bulk was increased by raw potato starch, green banana flour (RS_2), retrograded high-amylose maize starch and retrograded wheat starch (RS_3), and by fully digestible starch fed with wheat bran (control). Maize RS_3 did not alter fecal total SCFA concentrations from controls with respective mean values of 85.7 and 80.2 mmol/kg (74). If the data were to be expressed on daily output, these would diverge to 8.6 and 13.8 mmol/day, respectively. A similar difference emerges in the study by Holt et al. (142) where SCFA output increased with acarbose treatment while fecal SCFA concentrations remained unchanged. In the study by van Munster et al. (314), indices of fecal water cytotoxicity and colonocyte proliferation were lowered by the RS diet with no change in butyrate concentration. Total fecal excretion may be the more relevant measure, especially as greater fecal bulk is associated with lower risk of colorectal cancer. A contribution by RS to fermentation and not direct fecal bulking is supported by the increased excretion of bacterial dry mass from 6.3 g/day in controls to 11.3 g/day with acarbose (259). RS resembles highly fermentable NSP and OS and contributes to greater fecal microbial mass and SCFA. RS (as RS_2) may also increase fecal butyrate, but the effect of the other forms needs to be investigated systematically.

Only one published study has investigated effects of a high starch and RS diet (as opposed to specific foods containing RS). Total starch and resistant starch were increased by three- and sixfold with a simulated Chinese diet compared with a simulated Australian diet (211). This did not lead to putatively beneficial fecal outcomes, but the converse. Fecal mass (86 vs. 141 g/day) and SCFA concentrations were lower (Table 7), and fecal ammonia and phenols were higher on the Chinese diet (211). This study needs to be compared with that of Takahashi et al. (289), which showed that when Japanese subjects ate a similar high-starch diet, fecal SCFA were higher than on a self-selected diet (Table 7). The difference may reflect the relatively short duration of such interventions which last for 3–4 wk, which may be insufficient time for microbial adaptation. This adaptation is important for rice as fecal outputs and starch fall with time in pigs fed brown rice, a change attributed to the microflora (32). During the first week of feeding a simulated Indonesian diet, fecal wet weight and starch excretion were 365 and 2.5 g/day, respectively, when the diet was high in RS (as brown rice). Corresponding values were 247 and 0.8 g/day during *week 2*. Stool output (226 and 228 g/day, *weeks 1* and *2*) and starch excretion (0.8 and 0.8 g/day, *weeks 1* and *2*) stayed constant in pigs fed white rice at the same level of fiber, present as rice bran.

4. Animal studies

Animal studies have shown conclusively that feeding of RS of all types raises large bowel total SCFA. In rats, this has been shown through the feeding of potato, pulse, high-amylose corn, and chemically modified starches where cecal SCFA concentrations and cecal bulk and SCFA pools are increased. The role of microflora has been confirmed by the low SCFA values recorded in germ-free rats fed RS (12). Studies in pigs have shown similar results with greater large bowel SCFA concentrations and pools. Most studies show relative increases in butyrate, but an increase in propionate has been reported in rats fed a high-amylose maize starch (12) and in pig feces (43, 301). Greater fecal SCFA excretion has been shown in pigs fed RS (32, 43, 301). In keeping with the large bowel data, the increase was in butyrate excretion in pigs fed brown rice (32) and propionate in pigs fed high-amylose maize starch (43, 301). The latter also suggested that RS_2 could be fermented rapidly when compared with other types of RS. If so, this could be an important determinant of SCFA distribution along the colon. The effects of RS on total SCFA in these experiments are comparable to those of purified NSP and fiber-rich foods, while wheat bran is the product that raises butyrate consistently. However, there are data which show also that RS types differ and RS_3 may be more effective than RS_2 in promoting expansion of the biomass in pigs (134). A preliminary report has shown that starches acylated with a specific SCFA raise that acid preferentially in the large bowel of rats (13). This offers the opportunity to target delivery of individual acids to the colon.

D. NSP, RS, SCFA, Colonic Cell Proliferation, and Colorectal Cancer Risk

Further epidemiological studies have supported the earlier studies in Africans and shown that overall cereal consumption protects against colorectal cancer (139). However, evidence of a discrete benefit of fiber in colorectal cancer is not strong (139). Trock et al. (309) reviewed 39 epidemiological studies of diet and colorectal cancer risk and found that fiber intake was protective in only 50% of them. Of the latter, the effect achieved significance in only 45% of studies, conclusions similar to those of Cassidy et al. (53). Relative risk of colorectal cancer did not vary with energy-adjusted fiber intakes from 9.8 to 24.9 $g \cdot person^{-1} \cdot day^{-1}$ in a large (88,000) cohort of American women followed over 16 yr (113). Human intervention studies also have failed to show consistent effects. In one trial (The Australian Polyp Prevention Project), subjects were asked to follow a low-fat, high-fiber diet, but there was no difference in polyp recurrence compared with those eating a normal western diet, although the former diet inhibited the transition from smaller to larger adenomas (180). A recent intervention study in which patients were given a supplement of wheat bran fiber at 13.5 or 2 g/day showed no difference in the recurrence of adenomas with recurrence in 47% of the former and 51% of the latter (6). Kashtan et al. (154) fed wheat bran or oat bran to 45 polyp patients and 49 polyp-free volunteers and found no change in thymidine colorectal crypt-cell labeling before and after intervention. Whether patients with colorectal adenomas or carcinomas have enhanced proliferation is not established firmly despite several claims of positive association (82, 174, 292). A study using PCNA as a measure of proliferation found no differences between mucosa from normal patients and mucosa from patients with neoplasia (112). In ascending colon biopsies incubated in vitro, 10 mM sodium butyrate, ammonium butyrate, and butyric acid increased crypt labeling indices. The index was lowered with calcium butyrate, and deoxycholic acid-induced proliferation was abolished by sodium butyrate (26).

Starch is an important nutrient, and intakes vary widely across countries ranging from <150 $g \cdot person^{-1} \cdot day^{-1}$ in affluent westernized countries to >350 $g \cdot person^{-1} \cdot day^{-1}$ in societies consuming traditional agrarian diets (41, 53). Analysis of nutrient intakes indicates a negative relationship between total starch consumption and large bowel neoplasia (280, 296, 309). A meta-analysis of studies across 12 countries worldwide showed no relationship between dietary NSP and large bowel cancer risk ($r = -0.23$, NS, men and women combined) (53). In contrast, there was a strong inverse correlation ($r = -0.70$, $P < 0.001$) between starch intake and risk that extended to calculated intakes of RS ($r = -0.52$, $P < 0.05$). These data accord with the findings that low-risk populations such as the Japanese (161) or South African blacks (220) eat relatively little fiber, whereas high-risk populations (e.g., Australians) have quite high NSP intakes but eat relatively little starch (22). South African blacks ate only 43% of the recommended daily intakes of fiber but consumed substantially more maize starch than high-risk whites living in the same region (220). Fecal SCFA values were similar in both groups, but fasting and peak postmeal breath H_2 and CH_4 evolution was higher in blacks. The respective mean fasting values for both gases were 11.5 and 18.3 ppm in blacks and 6.2 and 8.0 ppm in whites. Rectal mucosal biopsies also showed lower epithelial cell proliferation in the blacks.

The protective mechanism of starch (or RS) is presumed to be through SCFA production due to its high fermentability. RS is trophic in rats as measured by greater cecal wall weight in animals fed RS_2 compared with animals fed a highly digestible starch (12, 125). However, data from pigs suggest that RS types may vary with longer colons in pigs fed RS_2 (301) compared with controls or animals fed RS_1 (32, 184, 303). However, the exact epidemiological relationship between these variables and

human colorectal carcinomas and adenomatous polyps is unclear due to the absence of systematic dietary RS and SCFA information. Data from cancer patients and interventions also are inconclusive. A brief report by Vernia et al. (319) indicated that fecal acetate was significantly lower in cancer patients (59.7 mM) than in those with polyps (79.6 mM) or in controls (89.2 mM). Butyrate concentrations were not significantly different between those with cancer (12.5 mM) and the other two groups (20.9 and 19.3 mM). Weaver et al. (322) showed that there were no consistent differences in SCFA between patients presenting for sigmoidoscopy for various reasons (including cancer) except for a higher molar contribution by butyrate in the controls (17.3%) compared with polyp/colon cancer patients (12.3 and 11.2%). Kashtan et al. (154) found no difference in fecal butyrate between controls and postpolypectomy patients except that fecal butyrate was lower in the latter following adaptation to a wheat bran supplement (16 vs. 6 mM). Thornton et al. (294) reported than in such patients ~6 g starch/day reached the colon as opposed to 10 g/day in controls. This could explain both the variable human SCFA data and the lower in vitro butyrate production rates by fecal inocula from these patients (65). However, this difference in starch digestion has not been confirmed (215). A decreased incidence of bowel cancer with lower pH has been reported (181, 320) but not confirmed (232). Upper crypt proliferative indices were lower in biopsies taken from humans consuming acarbose (in whom breath H_2 and fecal butyrate were raised) compared with those consuming placebo (142). ϕ_h was unaltered by treatment, but there was a strong negative correlation between butyrate levels and rectal crypt proliferation. Further support for lowered indices of risk came when consumption of RS_2 by humans lowered fecal secondary bile acid concentration (314). Colonic mucosal proliferation in rectal biopsies (measured with PCNA) decreased from 6.7 to 5.4%, while in vitro fecal water cytotoxicity also was lowered. The relationship between steroids, large bowel SCFA, and cancer risk may be important because secondary bile acids (such as deoxycholate) are thought to promote neoplasia. If lower bile acid excretion were to favor butyrate production and lower the cytotoxic potential of bile acids, there could be a stronger effect than for either change alone. RS increases fecal butyrate, and RS_3 lowers cholic acid excretion by 42% in human ileostomists (166).

Lower tumor numbers and/or burdens have been observed in rats treated with carcinogens when they were fed insoluble NSP such as cereal brans (107, 191, 192, 343, 346). These reductions were not necessarily related to large bowel SCFA. In contrast, enhancement of carcinogen-induced tumor growth has been seen with soluble and more fermentable fibers such as carageenan, pectin, guar, and alfalfa (27, 150, 240), although others have observed a decrease in tumors with guar and pectin (135, 325). In the latter study there was a correlation between butyrate production in the azoxymethane-treated rats and protection from tumors with hydrolyzed guar. In rats exposed to azoxymethane there was a significant reduction in the number of aberrant crypt foci with a raw potato starch diet (and also in energy intake) compared with a basic, sucrose, or cornstarch diet (295). Contrary evidence was found by Young et al. (343) who demonstrated hyperproliferation, greater density of aberrant crypt foci, and enhanced tumor production with dimethylhydrazine (DMH) using rats fed 20% of carbohydrate as raw potato starch. Wheat bran in addition to the potato starch reduced the enhanced tumor production but not the hyperproliferation. Sakamoto et al. (253) found no effect of resistant starch (10% by weight from high-amylose maize starch hydrolyzed with pancreatin) on tumor volume in the same model even though butyrate levels rose 1.5-fold in the cecum and 2-fold in feces. In these animals, cellulose halved cancer volume with no fecal SCFA changes, as was reported earlier by Heitman et al. (136). Cassand et al. (52) found that RS (type unspecified) reduced aberrant crypt foci in the DMH rat model. In this experiment there were large changes in fecal and cecal weight, with cecal butyrate being 2.6-fold higher and fecal butyrate 4.5-fold higher. In the Min mouse, most of the tumors are located in the small intestine, which raises questions about its relevance to humans. Despite this and the fact that it resembles only those humans who develop ~1% of total human colorectal tumors, its tumors are susceptible to cyclooxygenase inhibition (with sulindac) (35) as is human colorectal carcinogenesis (118). In Min mice, neither wheat bran nor RS (from retrograded high-amylose starch treated with amyloglucosidase) reduced colorectal tumors, although oligofructose did (226). However, Quesada et al. (233) have shown in the APC gene 1309 knockout mouse that acarbose treatment did not alter whole gut tumor multiplicity significantly. Gastric and large bowel tumor numbers were lowered by acarbose, but only the former was significant. Acarbose treatment decreased the number of tumors of diameter >3 mm from 3.78 (controls) to 2.36. Pierre et al. (226) examined effects of diet on intestinal tumors in Min mice and found most were in the small intestine and <7% in the large bowel. Neither wheat bran nor RS altered large bowel tumor numbers (2.1, 3.0, and 1.5 in control, RS, and wheat bran, respectively). Only OS reduced the number significantly to 0.7/mouse. These findings suggest that extrapolation from small intestinal tumors (which are very rare in humans) to colorectal tumors is not justified. This may apply also to the data obtained with carcinogens (AOM and DMH) because substantial numbers (0–50% of total, depending on diet) of tumors can occur in the small intestine (191), possibly reflecting reingestion of the carcinogen. There is also the possibility that such treatment could alter the microbial population and so influence the

results. Maciorowski et al. (179) showed that the injection of AOM altered the fecal bacterial population leading to higher anaerobe counts than controls when they were fed pectin. The opposite occurred with cellulose. The issue of the difference between rodents and other species may be involved because SCFA are associated positively with cecal but not distal colonic proliferation in rats (344).

Based on some of these negative or inconclusive studies, it has been suggested that the safety of RS may be doubtful (95, 98). This is a radical response to inconclusive animal data, especially given the epidemiological data showing negative associations between starch intake and colorectal cancer rates and human studies which show generally favorable changes in risk indices, including proliferation. The evidence from animal studies is more consistently negative for soluble fibers and cancer risk. Many of these NSP lower cholesterol by enhancing bile acid excretion (299), and Wasan and Goodlad (321) have even suggested that they may increase cancer risk in humans. Altered bile acid excretion could contribute to the data in rodents. In rats, RS_2 lowers plasma cholesterol through enhancing fecal steroid excretion (187), which would be expected to enhance experimental tumor formation. However, RS_2 does not affect plasma cholesterol in humans (153, 214) and actually lowers steroid excretion. On the basis that RS raises butyrate and lowers steroid excretion, it would not be expected to contribute to human colorectal cancer risk.

E. Potential Adverse Reactions: RS as a Malabsorbed Carbohydrate

By analogy with lactose, undigested starch could be regarded as a malabsorbed carbohydrate (13). It appears that in underdeveloped areas such as Myanmar (formerly Burma), starch malabsorption (measured through breath H_2 evolution) is common, with up to 70% of children being affected (34). In these areas, hygiene is inadequate and it appears that small intestinal bacterial overgrowth is a contributor to the syndrome, with 33% of rice malabsorbers being affected compared with 4.5% of absorbers (155). In contrast to malabsorbed lactose and similar sugars, there appears to be no adverse impact of RS on gastrointestinal function in well-nourished people with adequate food and personal hygiene. Moreover, in children with diarrheal disease, RS has been shown to promote recovery.

VIII. CONCLUSIONS AND FUTURE DIRECTIONS

Figure 3 provides an overview of the relationships between transit through the human gastrointestinal tract and the digestion of nutrients. Comminuted food (i.e., food that has been rendered more digestible by processing, chewing in the mouth, and wetting in the stomach) enters the small intestine where enzymatic digestion occurs. Unabsorbed food components enter the large bowel into a zone of high fermentative activity where SCFA levels are high. On passage of the digesta stream, fermentative substrate becomes depleted, and SCFA values fall due to absorption. Voided feces contain undigested food components, endogenous secretions, and biomass. Comparison of the actions of NSP and RS should be viewed in the context of this passage, which is a balance between passage and fermentability, which helps to explain why their effects are markedly different in some important respects. Fiber-rich foods, especially those high in insoluble NSP, are less fermentable than RS and are well-established laxating agents. Experimental studies have shown that they protect against chemically induced tumors in rodents. Paradoxically, the epidemiological data for a protective role for NSP in human large bowel cancer are weak, and any protection afforded at the population level is not great; water-soluble NSP may promote risk. In contrast, effects of RS on fecal bulking and laxation are inconsistent except when starch digestion is impaired specifically by acarbose. Some NSP and most RS are highly fermentable by the large bowel microflora, and there are data suggesting that RS fermentation favors butyrate production, but other results indicate that this is not uniformly true. The greatest difference between RS and NSP lies in relation to cancer, where the protection by RS in animals with chemically induced cancer is weak but the supportive epidemiological evidence is stronger. Indeed, it appears that some of the low-risk African populations that gave rise to the "fiber hypothesis" (67, 300) consume diets low in fiber but high in starch. If this is so, then large-scale interventions with high-starch (or high-RS) diets may be needed to ascertain if they are protective against cancer in patients at risk. Animal and human interventions with RS usually show improvements in indices of risk, while population studies associate greater starch (but not necessarily RS) intake with substantially lower disease incidence.

A major conclusion lies in the area of analytical technology that is well advanced for NSP with convenient enzymatic-gravimetric measures for fiber in which the foods are digested with enzymes (amylase, protease) to digest nonfiber components (17). Recent developments, including the application of liquid chromatography, offer to make this procedure more rapid (217). Interestingly, enzymatic-gravimetric methods measure a fraction of RS that is included in the overall "fiber" figure. This means that, depending on analytical procedure, some of the reported effects of fiber include a contribution by RS. Appropriate analyses (including steps to mimic mastication) have been developed for RS but need to be applied widely. Measures of intake at the population level remain inadequate, and it is a priority to develop the methodology further so that it can be applied widely. Any analytical

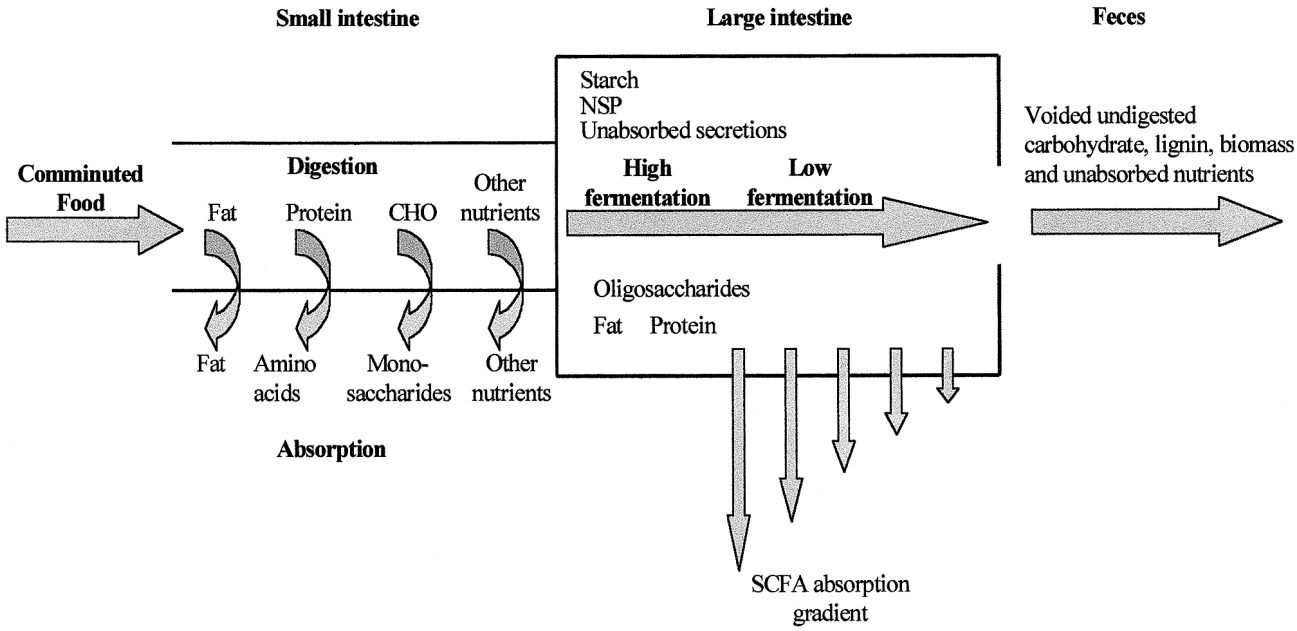

FIG. 3. An overview of the relationship between transit of food through the human gastrointestinal tract and the digestion of nutrients in the small intestine and fermentation in the cecum and colon. Foods are rendered digestible (comminuted) by processing (milling, cooking) and by mastication in the mouth. Digestion is also enhanced by wetting and macerating in the stomach. In the small intestine, digestion occurs through the action of intrinsic enzymes, and nutrients are absorbed. Food components and endogenous secretions not absorbed in that viscus pass through the ileocecal valve and are fermented. Fermentation is high in the proximal large bowel as is the SCFA production. Absorption of SCFA and of water and minerals (including calcium) is high in this viscus. On passage of the fecal stream, fermentation declines through substrate depletion, and SCFA values fall. The distal large colon and rectum are the regions of the large bowel with the most limited supply of SCFA and are the site of most pathology. Bacteria and unfermented components of low fermentability are voided in the feces.

procedure needs to accommodate the different types of RS, and only when this has been done will it be possible to assess how much RS is actually being consumed by animals and humans. Then, this will enable a realistic assessment of the relationships between RS, colonic metabolism, and disease risk.

The methodology to measure RS and NSP fermentation in vitro by digesta and fecal inocula seems to be established, and a major European collaboration has shown one way to maximize resources to determine SCFA production from different substrates and by different population groups. The factors that control starch fermentation and the products in vitro remain to be identified. Human experimentation is constrained by the ethical and logistic problems associated with accessing the human large bowel, so direct measures are difficult to obtain. Ileal starch digestibility can be determined in human ileostomists, but in situ determination of SCFA metabolism by intubation seems to be too difficult for routine application. Fecal measures of SCFA and other variables are valid but do not allow for continuous sampling and have yet to be applied at the population level. Indirect measures of SCFA production (breath gas evolution, peripheral blood SCFA) can be obtained in real time but are not very informative beyond indicating overall changes in fermentation. Animal experimentation remains necessary and, although rodents are used commonly, some of the data obtained appear of questionable relevance to humans. Other omnivores such as pigs and dogs seem to be more appropriate but pose ethical questions. Greater standardization of the experimental conditions is desirable.

A major deficiency is lack of knowledge of the relationships between diet, SCFA production, and the large bowel microflora, especially in relation to the distribution of SCFA in the colon and risk of disease. This is crucial because effects of RS appear to be largely through fermentation products and not physical bulking. Some types of RS can enhance production of specific SCFA, including butyrate, but the relationship needs to be defined more thoroughly. Current evidence suggests that a significant number of individuals lack the capacity to ferment RS_1, RS_2, and RS_3. Limited data indicate that rates of SCFA production and the molar ratios of the major acids may be characteristic of an individual and are not influenced by diet. These issues need to be clarified. Quantitative fecal cultures should be combined with measures of fermentation to elucidate which organisms are in low numbers or missing when some forms of RS (e.g., RS_3) are not fermented. The advent of newer, less labor-intensive tech-

nologies for bacterial identification and enumeration should enable this information to be gathered. There is no evidence that RS_4 (or any other form of RS) has any adverse effects on fermentation or large bowel physiology, and acylated starches have the potential to deliver specific SCFA to the colon. Through the production of SCFA, RS has specific benefits, e.g., management of infectious diarrhea, but the actual effectiveness needs to be established. This is especially relevant in the long-term prevention of serious diseases such as colorectal cancer. The suggestion that RS may increase large bowel cancer risk needs investigation, although it is not supported well by the epidemiological or human intervention data (which point to a protective role). Indeed, some of the doubts about RS and cancer risk may only be a consequence of the choice of experimental models (rats and mice). Other areas needing investigation include the transition from milk (breast or formula) to solid foods, especially adaptation to starches. It is not clear if low doses of resistant starch (10–20 g/day) affect colonic metabolism enough to modify disease risk. Clearly, dose-response studies are required, especially at higher levels of intake than those studied hitherto and whether any increase in intakes should be as total starch, RS or RS plus starch. Interventions (including the feeding of mixtures of RS of different types) would also show whether RS has a fecal bulking action at high levels of intake. Although recommendations have been made for fiber intake, it does not seem possible to do so for RS at present. In making such recommendations, it will be useful to make comparison between RS and NSP. This could be achieved by defining of RS in terms of "fiber equivalents" (67, 300), i.e., expressing actions of RS relative to fiber preparations of established effectiveness at known dose levels, e.g., wheat bran for bulking or isolates such as pectin for fermentation.

Drs. Tony Bird, Ivor Dreosti, and Ian Brown as well as Roger King made very helpful suggestions to the writing of this review. The contribution by D. L. Topping is in affectionate memory of Raymond A. Weller, who did a great deal to develop and sustain interest in this important subject.

Address for reprint requests and other correspondence: D. L. Topping, CSIRO Health Sciences and Nutrition, PO Box 10041, Kintore Ave., Adelaide BC 5000, Australia (E-mail: david.topping@hsn.csiro.au).

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