

Fermentation in the human large intestine and the available substrates^{1,2}

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Introduction

Fermentation, the process whereby anaerobic bacteria (and yeasts) break down dietary and other substrates to obtain energy for growth and the maintenance of cellular function, is an important component of normal large bowel activity. Evidence that fermentation occurs in man may be adduced from various observations. The human colon contains a rich and diverse bacterial population of predominantly saccharolytic species which synthesize many enzymes necessary for the catabolism of a wide variety of carbohydrates (1–3), the principal substrate for fermentation (Table 1). The major end products of fermentation, short-chain fatty acids (SCFA) (acetic, propionic, and butyric acids), are found in high concentrations in human colonic contents and are present in portal blood (4). Furthermore the net breakdown of carbohydrates in the colon, such as nonstarch polysaccharides which are not susceptible to normal digestive enzymes, has been adequately demonstrated in many studies (5).

The importance of fermentation to man, the host in this symbiotic relationship with the flora, lies mainly in its products and their fate in the body. SCFA, quantitatively the principal metabolites, are rapidly absorbed from the human large bowel (6). SCFA absorption stimulates sodium and water absorption (7, 8) and thus it is an important contributor to salt and water homeostasis in the colon. Colonic epithelial cells metabolize SCFA, especially butyrate, which is their preferred fuel (9–11). Butyrate also has important regulatory effects on nucleic acid metabolism in colonic cells through its capacity to inhibit histone deacetylase and so stabilize chromatin structure (12, 13). In cellular studies butyrate has been shown to be a differentiating agent (14) and so may be of importance in maintaining the health of the large bowel epithelium. Fermentation

is the only source of butyrate for this tissue. Once absorbed SCFA pass into the portal vein and thence to the liver where propionate and some acetate is taken up. Propionate is a gluconeogenic precursor in ruminant animals (15) but in hind gut fermentation may exert other effects (16–20). Acetate passes to peripheral tissues where it is metabolized by muscle (21, 22).

Other products of fermentation include branched-chain fatty acids from breakdown of branched-chain amino acids (valine, leucine, and isoleucine), ammonia, various carboxylic and phenolic acids, and amines. Ammonia may be incorporated into bacterial protein or absorbed depending on the amount of carbohydrate being fermented. The fate of the other substances is largely unknown at present although phenols arising from fermentation have been identified in urine. Active fermentation lowers luminal pH in the colon, which in turn affects the control of many enzymes, for example bile acid 7- α -dehydroxylase and amino acid decarboxylase.

Fermentation thus has an important role in human metabolism. However its control and the end products formed lie largely beyond normal regulatory and hormonal mechanisms which modulate other metabolic processes in the body because the enzymes crucial to this series of events are bacterial and *outside* the body in that they are not in contact with the circulation. The host does have some control over fermentation in that the colonic flora respond largely to the substrates available to them. The substrates which reach the large bowel can be regulated to a great extent by the composition of the human diet and the processing it undergoes before consumption.

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TABLE 1
Potential substrates for fermentation

A. Carbohydrate
1. Starch
2. Nonstarch polysaccharides (dietary fiber)
3. Unabsorbed sugars
4. Raffinose, stachyose
5. Polydextrose, Palatinit
6. Modified cellulose
B. Protein
1. Dietary
2. Endogenous (eg, pancreatic enzymes)
C. Other
1. Intestinal glycoprotein
2. Mucopolysaccharides

Dietary fiber (nonstarch polysaccharides)

Nonstarch polysaccharides (NSP) are principally the polysaccharides of the plant cell wall and include all non- α -glucan polymers in the diet such as cellulose, pectin, hemicellulose, etc. The observation that NSP are broken down in the human gut has led to studies of the importance of fermentation.

There are now many reports which confirm what was first noted over 100 yr ago, that NSP are digested in the human gut (5). The extent of breakdown varies somewhat as Table 2 shows. Results are all from studies conducted as balances with intake and output of fiber component being measured directly. Note that

widely different methods of analysis were used. In general NSP from foods are virtually completely degraded except for highly lignified sources such as bran. The site of this breakdown is the large intestine.

Table 3 gives the results of a series of experiments designed to measure the extent of breakdown of NSP in the human stomach and small intestine. People with an ileostomy (ie, having had their large intestine removed and the end of the small bowel brought out onto the abdominal wall as a fistula) were fed test meals of different NSP-containing foods and the amount recovered in the ileostomy effluent measured. Although it may be argued that such people are not good models for normal digestive physiology because their terminal ilea are more heavily colonized with colonic type microflora than normal subjects, they have in fact proved to be useful. Little or no evidence of fermentation occurring in the ileum is found. Notably there is a virtual absence of SCFA in the effluent (27, 28) and, as the results in Table 3 show, effectively no polysaccharide degradation. The NSP from wheat, oats, maize, banana, and potato resist digestion almost completely. In similar studies using the ileostomist as a model, Sandberg and colleagues show essentially complete recovery of NSP from pectin and wheat bran (30, 31). It is probably reasonable to conclude that all the NSP in the human diet reaches the large in-

TABLE 2
Breakdown of nonstarch polysaccharides (NSP) in the human gut

Source	Amount		% Digested (range)	Reference
	Fed	Recovered		
	g/d			
Wheat—cellulose	6.9	—*	29 (24–33)	Williams & Olmsted, 1936 (23)
—NCP†	14.3	—	35 (32–39)	
Cellulflower	10.1	—	7 (0–11)	Southgate & Durnin, 1970 (24)
Mixed diet (young men)				
—cellulose	8.0	6.8	15 (7–29)	
—NCP	13.5	3.7	72 (69–78)	
Mixed diet (young women)				
—cellulose	5.2	3.8	26 (6–40)	
—NCP	11.0	2.5	77 (73–84)	
Pectin	30.8	0.2	99	Cummings et al, 1979 (25)
Ispaghula	24.0	4.0	87	Prynne & Southgate, 1979 (26)
Banana—NSP	6.3	0.8	87 (85–92)	Englyst, 1985 (unpublished observation)
Potato—NSP	9.0	0.4	96 (94–99)	

* Value not given in paper.

† Noncellulosic polysaccharides.

TABLE 3
Digestion of nonstarch polysaccharides in the human small bowel*

Source	Amount fed	Amount recovered	% digested
	g	g	
White bread	2.3	2.3	0
Cornflakes	0.6	0.7	0
Oats	6.7	6.3	5.2
Banana	2.1	2.0	2.9
Potato	3.8	3.3	13.1

* From Englyst and Cummings (27–29).

testine. The amount available for fermentation can be determined simply by measuring NSP in food. This *simple* task has perplexed many analysts.

Measurement of NSP

Historically dietary fiber has been seen as an inert and indigestible material which has a negative effect in animal and human nutrition. Early attempts to measure fiber, such as the crude fiber technique (32), were based on harsh chemical treatments designed to isolate an indigestible fraction of the diet. Today there is substantial evidence that the polysaccharides, which comprise the major part of fiber, are by no means inert and affect digestive physiology in many ways, particularly through their breakdown in the large intestine. The fraction of the diet isolated in the crude-fiber technique bears little relation to the cell wall polysaccharides we know to be important in the gut (33) so there are now few people who support the use of this technique in human studies.

Two patterns of nutritional thinking have led to the development of other approaches to the measurement of fiber in food. One approach, arising from animal nutrition and following from the crude-fiber technique, has been to try and isolate gravimetrically (enzymatic-gravimetric methods) (34–37) a part of the diet that resists human digestive enzymes. Alternatively, we advocate attempts to define fiber in terms of its chemical constituents by methods derived from traditional carbohydrate chemistry (38–40). For analytical purposes it is now becoming accepted that fiber should be defined as the sum of NSP (non- α -glucans) (41–43).

The Englyst procedure (*Fig 1*) measures dietary fiber as NSP in plant foods. Starch is completely removed enzymatically and NSP is measured as the sum of its constituent sugars released by acid hydrolysis. The sugars may in turn be measured by gas-liquid chromatography (GLC), giving values for individual monosaccharides, or by a more rapid colorimetric procedure. A value is obtained for total dietary fiber and if required for soluble and insoluble dietary fiber. A small modification also allows cellulose to be measured separately (38, 39, 44, 45).

Using the Englyst procedure dietary fiber has been measured in more than 300 foods (unpublished observations) (46). Table 4 gives an example of the detailed information obtained with this procedure. Values are given for soluble, insoluble, and total NSP. These can be determined both by GLC and the more rapid colorimetric procedure (44). For routine use and quality control in industry, these data will often be sufficient. Detailed information about the composition of NSP with values for cellulose and the constituent sugars of NSP can however be obtained only by the GLC procedure. As will be seen from Table 4, the NSP of each plant material has its own unique spectrum of sugars. Detailed analysis by GLC gives an indication of the content of cellulose, pectin, β -glucan, and other polysaccharides. Such information allows more accurate predictions of the possible physiological role of the NSP from various foods.

The information obtained by the GLC procedure is essential not only in interpreting physiological studies but also for establishing food tables and in epidemiology where disease is related to type and amount of dietary fiber. Values obtained by the Englyst procedure are not affected by food processing so the dietary fiber content of various processed and mixed diets can be calculated simply from knowing the amount in the raw product. In a series of collaborative trials carried out in the UK (41, 45, 47), the Englyst method proved to be both accurate and robust and has been recommended for adoption for statutory purposes.

In recent years a fraction of starch made resistant to pancreatic amylase by food processing (resistant starch) was identified (38) and it has been suggested (48, 49) that it should be included as dietary fiber. Methods which



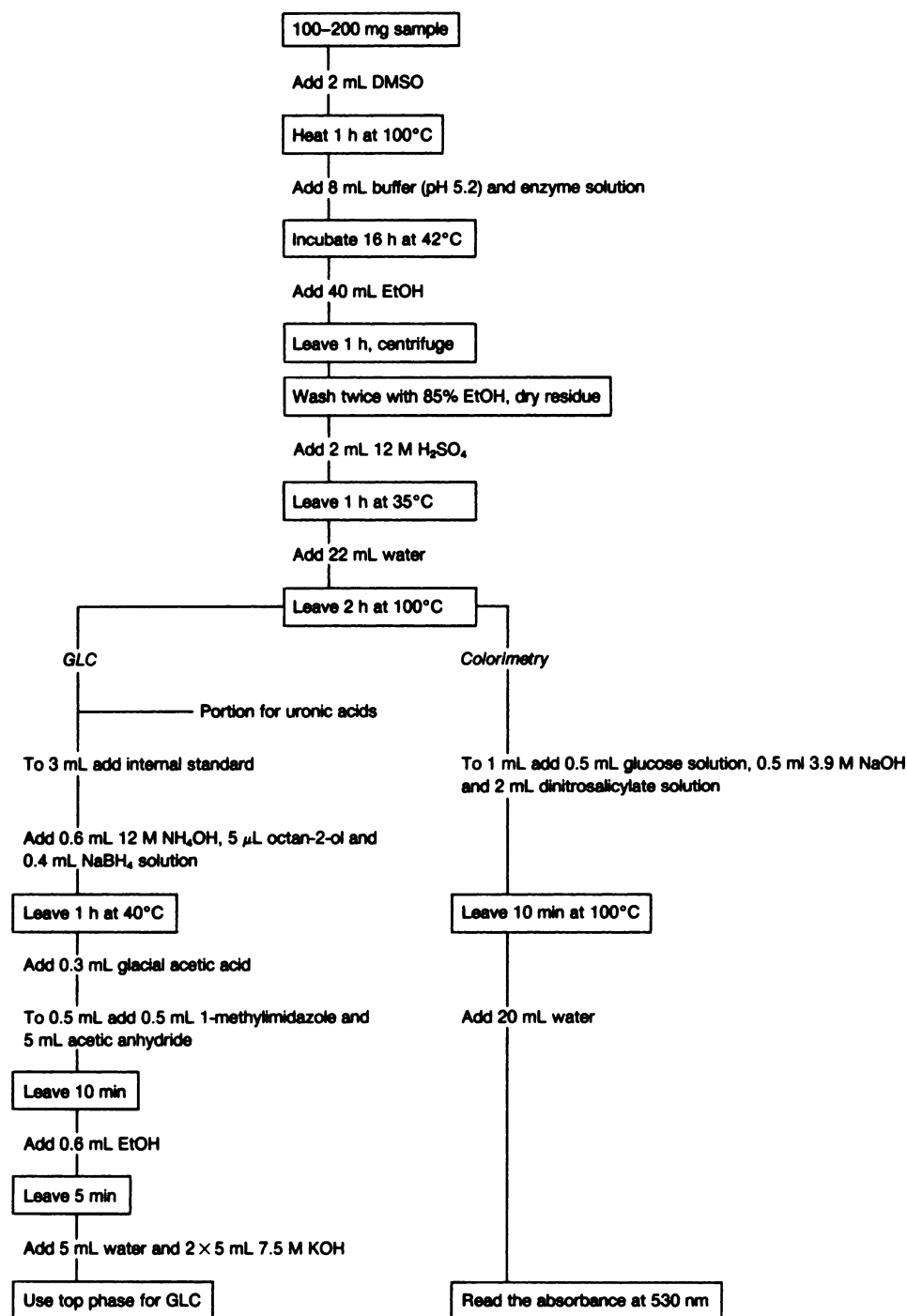


FIG 1. Procedure for analysis of nonstarch polysaccharides (NSP) by gas-liquid chromatography (GLC) and by colorimetry. From Englyst and Hudson (44). To measure soluble and insoluble NSP separately replace EtOH (40 mL) step with a 1 hr extraction at pH 7 in buffer.

TABLE 4
Nonstarch polysaccharides (NSP) in selected foods

Sample	Total g/100 g fresh weight	Total g/100 g dry weight	Composition g/100 g dry weight								
			Cellulose	Noncellulosic polysaccharides*							
				Rha	Fuc	Ara	Xyl	Man	Gal	Glu	UAc
Apples, Bramley											
Soluble NSP	0.6	4.4	—	0.1	t	1.1	t	t	0.3	t	2.9
Insoluble NSP	1.0	6.8	3.9	t	0.1	0.8	0.7	0.3	0.4	0.2	0.4
Total NSP	1.6	11.2	3.9	0.1	0.1	1.9	0.7	0.3	0.7	0.2	3.3
Bananas											
Soluble NSP	0.7	2.8	—	t	t	0.1	t	0.6	0.1	t	2.0
Insoluble NSP	0.4	1.7	1.0	t	t	0.2	0.2	t	0.1	0.1	0.1
Total NSP	1.1	4.5	1.0	t	t	0.3	0.2	0.6	0.2	0.1	2.1
Oranges											
Soluble NSP	1.4	9.8	—	0.3	t	1.9	0.1	0.1	1.4	0.1	5.9
Insoluble NSP	0.7	5.2	3.4	t	t	0.3	0.5	0.3	0.4	t	0.3
Total NSP	2.1	15.0	3.4	0.3	t	2.2	0.6	0.4	1.8	0.1	6.2
French beans, cooked											
Soluble NSP	1.3	12.7	—	0.2	t	1.1	0.2	0.2	2.6	0.1	8.3
Insoluble NSP	1.8	17.7	11.1	0.1	t	1.2	1.5	1.2	1.5	0.5	0.6
Total NSP	3.1	30.4	11.1	0.3	t	2.3	1.7	1.4	4.1	0.6	8.9
Garden peas, tinned											
Soluble NSP	1.1	5.7	—	0.2	t	3.2	0.2	0.1	0.3	0.1	1.6
Insoluble NSP	2.9	15.3	12.1	t	t	1.2	0.8	0.1	0.2	0.3	0.6
Total NSP	4.0	21.0	12.1	0.2	t	4.4	1.0	0.2	0.5	0.4	2.2
Winter cabbage, cooked											
Soluble NSP	0.9	15.8	—	1.1	0.2	3.2	t	0.1	2.2	t	9.0
Insoluble NSP	1.1	18.5	12.5	0.1	t	1.3	1.6	0.7	1.5	0.2	0.6
Total NSP	2.0	34.3	12.5	1.2	0.2	4.5	1.6	0.8	3.7	0.2	9.6
Carrots, cooked											
Soluble NSP	1.4	14.9	—	0.8	t	2.4	t	t	4.0	t	7.7
Insoluble NSP	1.1	11.1	8.8	t	t	0.4	0.4	0.5	0.6	0.1	0.3
Total NSP	2.5	26.0	8.8	0.8	t	2.8	0.4	0.5	4.6	0.1	8.0
Potato, old, cooked											
Soluble NSP	0.7	3.8	—	0.1	t	0.3	t	t	1.9	0.6	0.9
Insoluble NSP	0.5	2.6	2.0	t	t	0.1	0.1	t	0.3	t	0.1
Total NSP	1.2	6.4	2.0	0.1	t	0.4	0.1	t	2.2	0.6	1.0
White bread											
Soluble NSP	0.9	1.7	—	t	t	0.5	0.8	t	0.1	0.3	t
Insoluble NSP	0.6	1.1	0.2	t	t	0.3	0.4	0.1	t	0.1	t
Total NSP	1.5	2.8	0.2	t	t	0.8	1.2	0.1	0.1	0.4	t
Wholemeal bread											
Soluble NSP	1.2	2.3	—	t	t	0.7	0.9	t	0.3	0.3	0.1
Insoluble NSP	4.0	7.5	1.6	t	t	2.1	2.9	0.1	t	0.6	0.2
Total NSP	5.2	9.8	1.6	t	t	2.8	3.8	0.1	0.3	0.9	0.3
Cornflakes											
Soluble NSP	0.4	0.4	—	t	t	t	0.2	t	t	0.1	0.1
Insoluble NSP	0.5	0.5	0.3	t	t	0.1	0.1	t	t	t	t
Total NSP	0.9	0.9	0.3	t	t	0.1	0.3	t	t	0.1	0.1
Porridge oats											
Soluble NSP	3.6	4.0	—	t	t	0.2	0.2	t	0.1	3.4	0.1
Insoluble NSP	2.8	3.1	0.3	t	t	0.7	1.0	0.1	0.1	0.8	0.1
Total NSP	6.4	7.1	0.3	t	t	0.9	1.2	0.1	0.2	4.2	0.2

* Rha = Rhamnose, Fuc = Fucose, Ara = Arabinose, Xyl = Xylose, Man = Mannose, Gal = Galactose, Glu = Glucose, and UAc = Uronic acid.

measure dietary fiber and include starch are likely in the long term to lead to confusion and disputes. Starch which escapes digestion by pancreatic amylase in man may well be important in its own right (vide infra) but if starch is accepted as dietary fiber then manufacturers will be able to manipulate the amount of apparent *dietary fiber* in foods and so disguise the true value of dietary fiber as a food component.

Enzymatic-gravimetric methods, such as that of the AOAC (37, 50), aim to measure total dietary fiber defined as the "fraction of food . . . that is not digestible under the conditions of the test . . .", including the sum of the soluble and insoluble polysaccharides and lignin. The AOAC procedure, the development of which was based on a number of methods (35, 36, 38, 41, 51–53), uses enzymes to remove the majority of starch and protein. The residue is weighed and a correction applied for ash and residual protein. The residue contains some starch and a mixture of other substances including lignin, tanins, and Maillard products. It has been suggested that the residual starch which is included as dietary fiber in the AOAC (1985) analysis reflects the amount escaping digestion in the small intestine. This has not been shown for man and from a number of experimental studies it is now clear that much greater amounts of starch escape digestion in the small intestine than can be accounted for in the AOAC procedure. Thus the dietary fiber value obtained by the AOAC (50) procedure is not a measure of any definable component of the diet such as NSP, nor does it represent NSP plus starch escaping digestion in the small intestine of man. It does not give any information about either the amount or characteristics of substrates available for fermentation.

The amount of resistant starch (RS) and therefore dietary fiber measured by the AOAC procedure is dependent on the processing to which a food has been subjected. RS formation in a food is influenced by pH, water content, heating temperature and time, cooling, reheating, drying, and freezing (38). No RS is present in freshly cooked foods. However storage and pretreatment of samples for the AOAC and most other procedures results in a substantial amount of RS being formed. With the AOAC procedure, therefore, a single

source of food such as potato will yield a range of results for dietary fiber because of the different treatments the potato may have received. This makes the construction of food tables for dietary fiber difficult and the calculation of fiber content of individual dishes prepared from the same food or mixed diets impossible.

Lignin

Lignin is not included in our analytical definition of dietary fiber. Lignin is not a carbohydrate and should not be grouped analytically with NSP. It is a minor contributor to the human diet and with the exception of whole-grain cereals few foods contain measurable quantities. Furthermore it is difficult to determine chemically. None of the present dietary fiber methods which include a value for lignin can in fact justify this on strictly chemical grounds. These methods simply isolate a collection of material which is better referred to as *substances measuring as lignin*. The inclusion of an imprecise lignin value alongside accurate measurement of NSP is considered to be self defeating. Lignin should be measured separately as should be any other plant cell-wall-associated substance such as silica, cutin, etc that has been shown to be important in man.

Fermentation of NSP

An accurate and detailed method for determination of NSP is needed particularly for study of its fate in the large bowel. Using the Englyst method the average amount of NSP in the diet of groups of individuals is 10–18 g/d in the limited data published so far (54–56). Not all NSP behaves in the same way however during breakdown by large bowel bacteria. A wide range of polysaccharide-degrading enzymes are produced by colonic microorganisms (3, 57) and in vitro studies have shown that soluble polysaccharides such as pectin are broken down more rapidly than relatively insoluble ones like xylan. Free sugars do not accumulate in cultures of mixed organisms during fermentation indicating that polysaccharide degradation is the rate-limiting step in their conversion to SCFA. Time-course



studies have shown that side chains of branched molecules such as arabinogalactan are cointilized with backbone sugars and perhaps most importantly that the resulting pattern of SCFA produced depends on the substrate being fermented. From Table 5 it can be seen that all substrates produce acetate as the major end product in mixed cultures but the relative amounts of propionate and butyrate vary. Xylan and pectin lead to very little propionate and butyrate whereas arabinogalactan produces more propionate. In the same system starch fermentation leads to high amounts of butyrate formation (57). NSP are clearly degraded in vivo (Table 2) but major difficulties exist in identifying the amount and type of SCFA produced.

Starch

In the course of developing a method to measure dietary fiber as NSP, it was observed that processed foods such as white bread and cooked potato had higher apparent NSP content than the raw products (38). This seemed an unlikely possibility, and when detailed carbohydrate analysis was done of the NSP fraction it was apparent that the extra NSP was an α -glucan. This fraction, which resists dispersion in boiling water and hydrolysis with pancreatic amylase, was subsequently called RS (38). The possibility that starch which was resistant to α -amylase in vitro might also be resistant in the human gut and, therefore, reach the large intestine was raised. A number of studies were undertaken that showed that not only does RS largely escape digestion in the human small bowel but many other types of starch do also (27–31, 58–60) and that probably more starch than NSP reaches the colon.

TABLE 5
Short-chain fatty acids (SCFA) produced during fermentation of different polysaccharide substrates*

Polysaccharide	SCFA (mg/mg polysaccharide fermented)		
	Acetate	Propionate	Butyrate
Starch	0.25	0.13	0.21
Arabinogalactan	0.19	0.20	0.04
Xylan	0.42	0.10	0.02
Pectin	0.27	0.06	0.01

* From Englyst et al (57).

TABLE 6
Digestion of starch in the small intestine of man*

Source	Amount fed g	Amount recovered g	% digested
White bread	61.9	1.6	97.4
Oats	57.8	1.2	97.9
Cornflakes	74.2	3.7	95.0
Bananas	19.3	17.2	10.9
Potato			
Freshly cooked	45.4	1.5	96.7
Cooled	47.2	5.8	87.7
Reheated	47.2	3.6	93.4
Instant	48.0	1.3	97.3

* From Englyst and Cummings, (27–29).

Table 6 shows the results of a series of studies, using ileostomy subjects, of the digestibility of various starches in the small intestine. Cereal starches are very digestible; only a small fraction escapes into the large bowel. This fraction is largest for cornflakes and is very similar in amount to the RS content of this food.

Banana however contains no RS, its starch being easily digested by pancreatic amylase after gelatinization. However most of the starch in banana reaches the large intestine. This is probably because in raw banana the starch in the granules is in a highly crystallized form, x-ray diffraction type B as described by Katz (61), which largely resists α -amylase. In oats, another food eaten virtually raw, the crystalline structure of the starch granules is less compact (x-ray diffraction pattern A), allowing access by α -amylase. Gelatinized starch is usually readily digestible as can be seen with freshly cooked potato (Table 6). If cooked potato is allowed to cool however, > 12% (5.8 out of 47.2 g) of the starch escapes breakdown in the small bowel. This is over four times the RS content in cooled and cooked potato. Thus a measurement of the RS content of a food will not reflect the amount of starch escaping digestion in the small intestine. Other forms of starch, quantitatively more important, are also not absorbed.

As a result of these studies a nutritional classification of starch has been proposed (62) and is outlined in Table 7. On the basis of physical form and susceptibility to pancreatic amylase, starch may be classified into readily

TABLE 7
Nutritional classification of starch*

Type of starch, based on susceptibility to pancreatic amylase in vitro	Physical form	Present in	Predicted fate in the small intestine of man
Readily digestible starch (RDS)			
RDS (1)	Dispersed starch	Freshly cooked food	Rapidly digested
RDS (2)	Crystalline starch with X-ray diffraction pattern A	Most uncooked cereals and gelatinized starch dried at high temperature	Slowly but virtually completely digested
Partially resistant starch (PRS)			
PRS (1)	Physically inaccessible starch	Whole or partly milled grains and legumes	Partly digested
PRS (2)	Starch granules with X-ray diffraction pattern B or C	Raw potato and banana	Partly digested
PRS (3)	Mainly retrograded amylopectin	Cooled cooked potato	Partly digested
Resistant starch (RS)			
RS	Mainly retrograded amylose	Cooled cooked potato, bread and cornflakes	Resists digestion totally

* From Englyst and Cummings (62).

digestible starch (RDS 1-2), partly resistant starch (PRS 1-3), and resistant starch (RS). Thus the long-held assumption that starch is completely hydrolyzed in and absorbed from the small intestine is invalid. This residual starch can be fermented with the production of SCFA and effects on bacterial growth (63). However there are marked differences between starch and NSP both in the small bowel and the large, where it is fermented like other polysaccharides at a rate and to end products that are characteristic of this particular group of polymers.

The amount of starch reaching the large intestine is clearly of major importance in determining many aspects of fermentation and colonic function. It is not possible to give a figure at present but a number of things need to be taken into account when making an estimate.

Table 8 lists those factors which, mainly in studies of blood glucose and insulin, have been shown to affect starch digestibility in man. As a result of these studies the general hypothesis has emerged which proposes that by alteration of the type of starch in the diet a benefit to health can be achieved through reducing blood glucose and insulin response to food. The importance of the rate and extent of starch di-

gestibility in the small intestine to large bowel function has not really been explored. Nevertheless the factors listed in Table 8 may be of importance also in determining not only carbohydrate metabolism but colonic function.

Because the rate of starch breakdown, rather than the amount escaping digestion in the small bowel, has been the main concern for most investigators, few have quantitated small bowel losses. However, Levitt's group in a series of studies using breath hydrogen as a marker of carbohydrate malabsorption have calculated that 10–20% of the 100 g of carbohydrate in a meal of white bread is not absorbed, 8% in oat bread, 6% in corn bread, 13% in potato, and 18% navy beans (60). When contrasted with the data in Table 6, which are derived from direct measurements using ileostomy subjects, the breath hydrogen technique seems to give higher values for starch malabsorption. In ileostomy studies Chapman et al (59) found only $2.4\% \pm 1.3$ (SD) (1.9 g) of starch malabsorbed from meals of wheat and potato starch. However, Wolever et al (83), who found total carbohydrate passing to the large intestine after test meals of 11% (9.5 g) from white bread, 8% (7.7 g) wholemeal bread, and 18% (20.8 g) red kidney

TABLE 8
Factors which may affect starch digestion in the human small bowel

Description	Reference
Granule structure	Katz et al, 1937 (61) Booher et al, 1952 (64) Englyst & Cummings; 1985, 1986 (27, 28)
Amylose:amylopectin ratio	Goddard et al, 1984 (65) Wolf et al, 1977 (66)
Physical form	Crapo et al, 1977 (67) Haber et al, 1977 (68) O'Dea et al; 1980, 1981 (69, 70) Coulston et al, 1980 (71) Snow & O'Dea, 1981 (72) Wong & O'Dea, 1983 (73)
Amount in diet	Stephen et al, 1983 (58) Chapman et al, 1985 (59)
Transit time	Chapman et al, 1985 (59)
Food processing	Collings et al, 1981 (74) Jenkins et al, 1982 (75) Thorne et al, 1983 (76) Englyst & Cummings; 1985, 1987 (27, 29)
Amylase inhibitors	Puls et al, 1973 (77) Layer et al, 1985 (78) Levitt et al, 1984 (79)
Presence of NSP	Wolever & Jenkins, 1986 (80)
Lectins	Rea et al, 1985 (81)
Phytate	Yoon et al, 1983 (82)

beans, found that breath hydrogen closely reflected the findings in ileostomy subjects. In studies in healthy people intubated so that direct measurement of carbohydrate passing through the ileum could be made, Stephen et al (58) found 6 and 9% (2.1 and 4.8 g) of the carbohydrate from two mixed meals was malabsorbed.

Despite these discrepancies it is clear that significant starch malabsorption has consistently been found and that in many meals the amount will exceed several-fold that of NSP. On a western diet significant amounts of starch may pass into the large intestine of man. In countries where starchy foods are the main part of the diet, eg, South India (rice), Uganda (banana), South Africa (maize), and where food is often eaten cold (which will result in starch resistant to α -amylase), then substantially more may be passing into the colon.

Fermentation of starch

Human feces contain high levels of amylase activity, most of which (92%) is extracellular (84). However bacterial-cell-bound amylase are much more effective at breaking down starch than are the soluble enzymes in cell-free supernatants from feces. The predominantly amylolytic bacteria belong to the genera *Bifidobacterium*, *Bacteroides*, *Fusobacterium*, and *Butyrivibrio*. Most of the soluble amylase activity in feces is probably derived from human pancreatic enzymes since pancreatectomy reduces total activity in feces to < 3% of that found in healthy individuals (85). Nevertheless fecal flora are able to utilize starch with the production of SCFA.

Cooking and processing of starchy food-stuffs renders part of the starch resistant to mammalian α -amylase. However RS is completely degraded by washed bacterial cells obtained from human feces although the cell-free supernatants from these feces, containing predominantly host-derived amylases, do not degrade RS (63). When hydrolysis by bacteria of RS and digestible starch are compared then it may be seen that digestible starch is fermented more rapidly. As a consequence of this there is an initial accumulation of oligosaccharides and lactate. Ultimately however SCFA are the main end products. RS is broken down more slowly, oligosaccharides and lactate do not accumulate, and the amount of SCFA produced is less; 54% of the carbon in readily digestible starch is converted to SCFA while the corresponding figure for RS is only 30%. No qualitative differences occur between RS and digestible starch in end products but as was noted in Table 5, starch fermentation is associated with greater production of butyrate than fermentation of NSP.

Other substrates

Many other substrates are potentially available for fermentation in the human colon (Table 1). Mostly they contain carbohydrate in some form. Dietary lactose will pass into the large intestine in people with β -galactosidase deficiency and will be fermented since this enzyme is widely present in bacteria. Lactose is rarely found in adult feces. Sucrose and other sugars may be malabsorbed occasionally,

for example in patients with jejunio-ileal bypass. Rapid fermentation again occurs (86). The production of hydrogen after the ingestion of the trisaccharide raffinose and tetrasaccharide stachyose again indicate fermentation of these sugars, which are found mainly in beans (85, 87, 88). Food manufacturers are now developing a range of carbohydrates that do not occur naturally but that can be incorporated into food to improve its texture and other properties. These include modified celluloses such as methyl-, carboxymethyl-, ethyl-, etc, the fate of which is largely unknown in the human gut. Polydextrose (a random polymer of glucose) and Palatinit (a synthetic disaccharide) are both partly fermented (89, 90).

Together these substrates account for only a small fraction of total fermentable material available to the flora. In subjects consuming western diets, the amount of dietary carbohydrate other than starch and NSP is unlikely to be > 10 g/d and is more probably ~5 unless the diet contains unusual quantities of beans or the subject is drinking milk and lacks intestinal β -galactosidase. However this may change with the increasing use of modified and synthetic carbohydrate in food processing.

Mucus degradation by colonic microflora has been well documented (91, 92) as has the breakdown of the mucopolysaccharide chondroitin sulfate (93) and probably adds a further 3–5 g for fermentation.

Protein

The importance both quantitatively and metabolically of protein as a substrate for fermentation in the human colon is only just emerging. The human large intestinal contents exhibit substantial proteolytic activity, partly attributable to pancreatic enzymes, but which can also be shown to be a property of a number of colonic microbial species. The predominant proteolytic bacteria in man are *Bacteroides* spp and *Propionibacterium* spp, present at 10^{11} – 10^{12} and 10^8 – 10^{10} /g dry feces, respectively (94). Other proteolytic species include *Streptococcus* and *Clostridium*. In vitro studies with human fecal flora show that proteolysis occurs rapidly and that peptides and amino acids do not accumulate, instead SCFA, ammonia, and various phenolic acids are produced. The hu-

man colon contains measurable quantities of the branched-chain fatty acids, isobutyric acid, and isovaleric acid (4), which arise from the breakdown of valine and isoleucine. Amino acid fermentation is thus occurring and the rate-limiting step in this process is degradation of protein itself.

The amount of protein entering the human large bowel can be partly deduced from studies of ileostomy subjects. On protein-free diets ~1 g N/d is lost in ileostomy effluent (95). This rises to ~2 g/d when normal food is taken and is influenced both by the amount of protein in the diet (96) and the physical form of the food (95). The majority of the N is protein (48–51%) and peptides (20–30%). On N-free diets protein losses comprise mainly pancreatic enzymes while with normal food amino acid patterns in ileostomy effluent suggest that food residues are the major contributor. Surprisingly the amount of NSP appears not to influence small-bowel protein losses (30, 95) except when purified polysaccharides such as pectin are given (31).

Conclusions

Fermentation, the process whereby energy yielding substrates are broken down by anaerobic bacteria, is the essence of normal large bowel function in man. Its control is dependent mainly on the amount and type of substrate available to the flora since this will determine the magnitude and rate of many metabolic events in the colon. Many substrates, both dietary and endogenous, are available for fermentation but in the present state of knowledge the principal ones are probably starch, NSP (dietary fiber), and protein. All dietary NSP reaches the colon but the amount of starch and protein that does is dependent on the processing which food has undergone before ingestion. In western countries the amounts of fermentable material are on average 12 g NSP, 12 g protein, and an unknown quantity of starch and other carbohydrates, with a wide range for total substrate available to the flora. In countries where starchy foods, such as rice, maize, plantain, etc, are the main contributors to the diet then the amount fermented may be greater. The end products of fermentation, chiefly short- and branched-



chain fatty acids, various gases, phenols, ammonia, and amines, are of physiological importance and will vary according to the substrate available. It is important in understanding large bowel function that these fermentable dietary components are measured separately and exactly. An accurate and detailed method for NSP is available and a new classification of starch for nutritional purposes is proposed which takes account of the site of its breakdown in man. Problems in quantitating protein and endogenous substrates available for fermentation remain to be solved.

The large intestine is thus an important and hitherto neglected digestive organ in man. An understanding of fermentation is likely to be important in unravelling the problem of large bowel and possibly other diseases. 

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