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NUTRITIONAL ROLE OF RESISTANT STARCH: Chemical Structure vs Physiological Function

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INTRODUCTION

Starch is second only to cellulose in abundance as one of the main carbohydrates synthesized by plants. Starch also represents the primary energy source for many animals, including humans (35). Historically, starch has occupied a

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central role in human nutrition. Starch-based plant foods have been major staples in many cultures, and only recently in affluent developed countries have they been displaced by foods high in fat and/or protein (100).

In some senses, the perceived role of dietary starch has been a rather negative one. Dietary starch has been viewed either as a replacement for dietary fat or as a reference carbohydrate in nutritional studies. Differences between starch and simple sugars were thought to lie solely in the relatively slow digestion of the former. This concept of slow hydrolysis underpins the glycemic index whereby excursions in peripheral venous blood glucose concentrations in response to various carbohydrate foods are expressed relative to a suitable control such as glucose (43). Starchy foods generally (but not always) yield lower glycemic index values (95). Consequently, starch hydrolysis varies from quite rapid to very slow. It is becoming appreciated that small intestinal starch digestion may be so retarded that starch can escape into the large bowel. This fraction has earned the name resistant starch, and the factors that control it are giving a whole new direction to research in dietary complex carbohydrates. These factors include the physical and chemical attributes of either the starch or the whole food as well as the physiological consequences of starch entry into the large bowel.

STARCH STRUCTURE

Primary Structure of Starch Components

In plants, starches are packaged in granules. Following disruption and dissolution of these granules, most starches can be fractionated into two components—amylose and amylopectin (35). Both of these macromolecules are glucose homopolysaccharides (Figure 1). Amylose is an essentially linear molecule in which the D-glucose units are linked by α-(1 → 4) glycosidic links. It is polydispersed with a range of molecular weights that depends upon

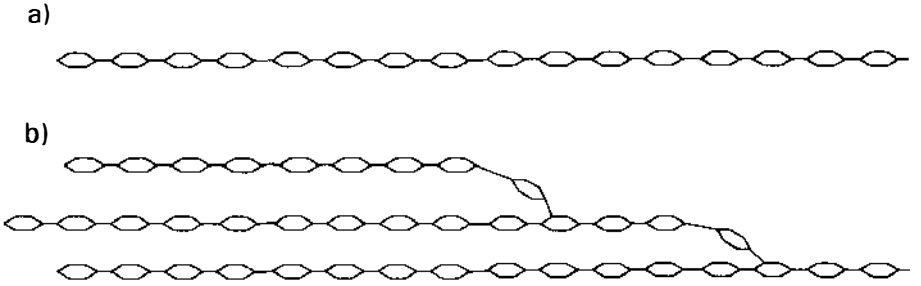

Figure 1 Schematic of (*a*) amylose and (*b*) amylopectin illustrating their essentially linear and branched structures, respectively.

source. Gallant et al (29) reported that amylose contains 500–600 amylose glucose units (AGU).

β-Amylase cleaves the penultimate glycosidic link from the reducing end of the polysaccharide to release maltose (4-O-α-D-glucopyranosyl-D-glucopyranose). When isolated amyloses are treated with β-amylases, digestion is often incomplete. These barriers to digestion are overcome by incubation with other amylolytic enzymes, such as pullalanase or isoamylase, thus indicating the presence of α-(1 → 6) linkages in the amylose.

Similar studies have revealed that amylopectin contains both α-(1 → 4)-linked glucose and a number of α-(1 → 6) linkages. Approximately 5% of the glucose residues may be joined in this manner, thereby yielding a more branched structure. Amylopectin consists of short (12–70 residues, average 20) α-(1 → 4)-D-glucan chains linked by occasional α-(1 → 6) bonds. The amylopectin molecule is very large, with a molecular weight in excess of 10^7 daltons, but its exact structure remains unknown. Studies with debranching enzymes and β-amylase point to three distinct populations of glucan chains containing 12–20, 40–45, or > 60 glucose residues (29). Amylopectin is the major component of most starches, the composition of which can vary from virtually pure amylopectin (e.g. in waxy barleys and rice) to high levels (≥ 70%) of amylose [e.g. in the wrinkled pea (*Pisum sativum*)] and in amylomaizes.

Structure of Starch Granules

The size and shape distribution of starch granules varies among plant species and even among cultivars. In general, granules from tubers (potatoes, yams, etc) are large and spherical, whereas those from cereals are small and polyhedric, and legume starch granules are kidney shaped (29). Starches from different sources can be distinguished by physical methods such as polarizing microscopy that assess the order that amylose and amylopectin possess in the granule. Such techniques suggest that the granule is composed of a large number of small, randomly orientated crystalline regions in an amorphous matrix with little or no order (27). The crystallinity of native starches falls in the range of 15–45% (104). X-ray diffraction analysis of native starches yields two types of spectral patterns, A type and B type, which points to two types of crystalline structures. Cereal starches yield the A-type pattern, whereas tuber starches and amylose-rich starches yield the B-type pattern. Legume starches yield an intermediate (C-type) pattern, which appears to be a mixture of A- and B-type patterns rather than a distinct crystalline structure (30). The molecular arrangements of the α-glucan chains that give rise to the A- and B-type patterns are very similar to the α-glucan chains forming left-handed, parallel-stranded double helices with a unit cell length of 12 AGU (39, 40). The packing of the helices differs, as does the amount of associated water. B-type starches hold more water than A-type starches.

The factor that most likely influences the pattern in plants is the length of

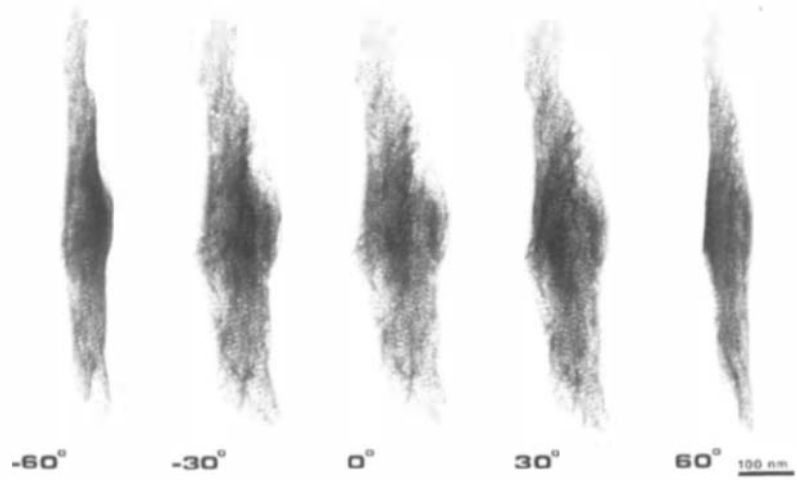

Figure 2 Electron micrographs of negatively stained starch fragments showing amylopectin. Reprinted from Ref. 64, with permission.

the amylopectin chains, which are shorter in A-type starches (97). Both amylose and amylopectin can form crystalline structures, and the α-(1 → 6) branch point of amylopectin favors helix formation. However, these crystalline structures are probably mostly amylopectin because the steeping of starch granules in water leaches out amylose, leaving both the amylopectin and the crystallinity intact (105). Oostergetel & van Bruggen (64) examined potato starch using electron optical tomographic and cryo-electron diffraction techniques. They constructed a model of the arrangement of amylopectin in the granule in which the helices of amylopectin form a continuous network in the granule and the α-(1 → 4) glucan portion of the molecules are crystalized into 5 nm-wide lamellae, with the α-(1 → 4), (1 → 6) portion forming the amorphous region in between (Figures 2 and 3).

When heated in the presence or absence (annealing) of moisture, the A and B diffraction patterns are lost. A new V pattern develops as a result of the formation of a complex in which the amylose forms a helix around fatty acids. Similar helical structures can form around iodine. These structures yield the characteristic blue color of starch-iodine complexes. Occasionally the V pattern occurs in native starches with an amylose level above 30%.

MODIFICATION OF STARCH STRUCTURE DURING FOOD PROCESSING

Very little raw starch is consumed in normal diets, and processing almost invariably involves the application of heat (and moisture) for varying lengths

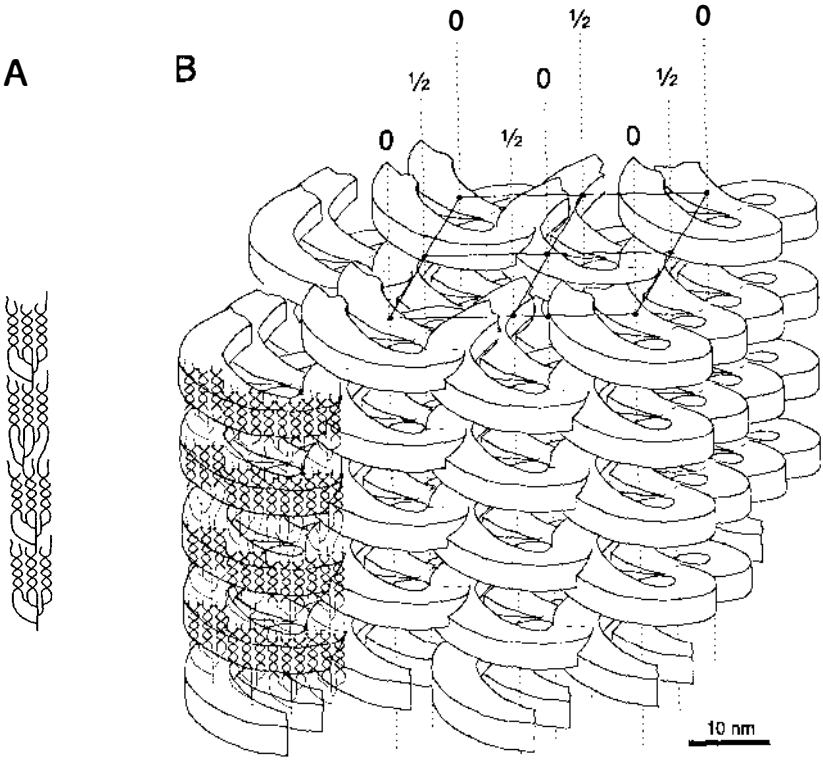

Figure 3 Model for the arrangement of amylopectin in potato starch. Reprinted from Ref. 64, with permission.

of time. This process disrupts the starch granule, which leads to the breakdown of the A- and B-type crystalline forms. The effect of processing on the structure of starch in the food can vary considerably, affecting the digestion of the starch and thus its nutritive value.

Heat/Moisture Treatment

When starch-containing foods are heated at low to moderate levels of moisture, the crystalline structures within the starch granule lose order (although the granule itself may retain its shape). This disordering is referred to as melting and occurs at temperatures that vary with moisture content and with the origin of the starch. In the absence of water, melting temperatures exceed 150° C but fall to 100–120° C at 20% moisture. The latter are the temperatures for making biscuits and other baked products. Studies of the melting of model A- and B-type crystalline amylose spherulites revealed that at moisture contents above

40%, the A type melted at temperatures 20° C higher than the B type (97). Generally, however, A-type starches do not melt at higher temperatures than B-type starches, which probably indicates that factors in the amorphous matrix of the starch influence the melting of the crystallites.

When starch is heated in an excess of water, gelatinization occurs as a two-stage process. The first stage involves the disruption of the crystallites and takes place at temperatures of 60–70° C (11). Loss of birefringence and of A and B patterns is discernible by X-ray diffraction. Scanning electron microscopy studies of potato and corn starch have revealed a honeycomb-like structure that appears in the granules as they gelatinize. Above 90° C, a marked loss of granular structure occurs, although the starch granules may remain as fragments comprised of amylopectin suspended in a solution of amylose (44).

The response of starches to heating in different moistures varies with the type of starch. Granules high in amylose swell more slowly than those rich in amylopectin, and differential scanning calorimetry reveals changes that reflect a loss of order within granule, followed by its destruction (11). However, the disordering is a stepwise process in which the glucan chains lose their double helical structure and become coils. In some starches with high levels of associated fat, the coils can complex with different lipid fractions. Further heating leads to disruption of these complexes, and more random orientations are adopted by the molecules. In most food systems, some order of the starch molecules is likely always to be present. Order is very important, not only because it imparts texture to the food, but also because of its inevitable effects on starch digestion when the food is consumed.

Gelation, Retrogradation, and Resistant Starch Formation

Almost all processed foods undergo a period of storage of variable length at moderate or low temperatures before consumption. During this period, further changes in starch structure can occur. When moisture is present and gelatinization of the starch has occurred, the amylose and amylopectin molecules can associate to form a gel (59). The exact nature of the gel depends on variables such as the amylose:amylopectin ratio, the amount of water present, and the time and temperature of storage. The network begins as the glucan chains re-adopt a helical structure; the helical structures then associate to form junction zones to establish an extended network of polysaccharides. Starch gels are unstable and age with time as crystallinity within the gel increases. This crystallinity can be detected by X-ray diffraction and displays a B-type pattern (10). In the presence of fatty acids and monoglycerides, a V-type pattern is observed as the amylose chains complex with these species.

B-type crystallinity is referred to as retrogradation and may take from several hours (in the case of high-amylose starch) to several days (in the case of high-amylopectin starches) to form. Following an extensive investigation of

the retrogradation of acid-treated wheat starches, Zhang & Jackson (103) concluded that retrogradation rates were greatest in starches with smaller amylopectin fragments with fewer branch points and narrower linear chain-length distributions. Retrogradation can be increased by repeated heating and cooling cycles (75). This process encourages formation of the extended crystalline regions of α-glucan.

Extensive treatment of starch subjected to retrogradation processes with α-amylases breaks down nonretrograded starch, leaving a fraction that resists amylolytic degradation and that has been termed resistant starch. Russell et al (70) examined retrograded wheat starch after extensive purification procedures to remove protein and lipids. They established that resistant starch is composed of almost pure linear α-glucan chains of degree of polymerization (DP) ~ 60. Starch from a waxy maize yielded a fraction composed of α-glucan chains of DP ~ 40, which reflects the shorter chain lengths of amylopectin compared with amylose. Resistant starch prepared from wheat consists of linear glucans of DP ~ 65 (78). Differential scanning calorimetry (DSC) analysis revealed that the fraction was crystalline in nature, and X-ray diffraction showed that it had a B-type structure. However, resistant starch with an A-type structure can be isolated if the starch is held for extended periods at high temperature after gelatinization (16). Sievert & Wursch (77) studied the effects of repeated heating and cooling on potato amylose (DP 5500) and resistant starch (DP 65) isolated from maize starch. They reported that during the heating part of the cycles, the amylose samples showed melting transitions at 153.6° C and 139.8° C, respectively. On cooling, the chains of amylose reassociated at 80–70° C and 60–55° C. Reassociation was inhibited by fast cooling rates (> 10° C/min) for high molecular weight amylose, but not for low molecular weight amylose.

Retrogradation and the formation of resistant starch occurs more readily in starches with higher levels of amylose (76) because long chains of α-glucan are needed to form the crystalline structures. However, retrogradation and resistant starch formation can also occur when waxy starches devoid of amylose are heated and cooled. Yuan et al (102) examined amylopectin structure in two lines of three genotypes of waxy maize starches. One genotype had amylopectin with a greater proportion of α-glucans with chain length DP > 30. DSC examination of their retrograded starches revealed higher melting temperatures and enthalpy changes associated with the loss of crystallinity, which indicates that the formation of enzyme-resistant structures is likely dependent on the DP of amylopectin and amylose.

The changes that occur in the structure of starches during heating and cooling have been studied extensively because of their profound influence on the functional properties of foods. However, this processing is of considerable nutritional importance because it influences starch digestibility in the gastrointestinal tract.

STARCH DIGESTION

Starch must be completely depolymerized to glucose before it can be absorbed in the small intestine. Depolymerization is effected by several digestive enzymes that cleave the α-(1 → 4) and α-(1 → 6) glucosidic bonds. In monogastric species, the main enzymes are α-amylases [(α-1 → 4) glucan hydrolase, EC 3.2.1.1.)] (33). When incubated with amorphous or highly dispersed starch, they act on both amylose and amylopectin in an endo fashion, releasing glucose, maltose, and higher dextrins (glucose polymers, DP1-7) (75). The glucose is absorbed directly through the intestinal mucosa, whereas the oligosaccharides are acted upon by membrane-bound glucosidases. These enzymes include glucoamylase, a hydrolase that cleaves α-1,4-glucan links in extended α-glucan chains, thereby removing successive glucose units from the nonreducing ends. In addition, this enzyme hydrolyzes maltose to glucose. Maltose and its higher oligosaccharides are cleaved by the sucrase α-dextrinase complex (also termed sucrase isomaltase). The sucrase cleaves α-(1 → 4) glycosyl oligosaccharides and splits sucrose into glucose and fructose.

Some evidence from intubated humans indicates that free glucose is found in the terminal ileum, which points to its probable passage into the colon (82). However, starch itself can pass into the large bowel. Nutrition scientists have termed this fraction that escapes digestion resistant starch—an unfortunate choice because it suggests identity with the starch resulting from retrogradation. Obviously, retrograded starch will resist amylase attack in the small intestine, but the resistant starch of nutrition scientists is the result of additional factors. One must distinguish carefully between chemically resistant starch (i.e. enzyme-resistant starch in vitro) and physiologically resistant starch (i.e. starch that passes undegraded through the small intestine and into the large bowel). Englyst et al (18) classified resistant starches according to which factors are intrinsic to the food or to the processes used in its preparation (Table 1).

Table 1 Classification of resistant starches[1]

Type of starch	Example of occurrence	Probable digestion in the small intestine
Rapidly digestible starch (RDS)	Freshly cooked starchy foods	Rapid
Slowly digestible starch (SDS)	Most raw cereals	Slow but complete
Resistant starch		
1) Physically indigestible starch	Partly milled grains and seeds	Resistant
2) Resistant starch granules	Raw potato and banana	Resistant
3) Retrograded starch	Cooled, cooked potato, bread, and cornflakes	Resistant

[1] From Ref. 18.

Intrinsic Factors and Digestibility

PHYSICAL BARRIERS Food particle size can affect starch digestion by amylases as a result of surface area because smaller particles (which have a larger surface area relative to volume) are digested more rapidly than larger ones. In recent years, the move toward food products such as whole grain bread that contain larger particles has introduced fractions into the diet that may be relatively indigestible. A recent study compared the digestibility of starch in bread containing whole kernels of wheat, rye, barley, and oat groats in humans and in vitro with that of bread made from barley and wheat flours (51). The glycemic index was used as a measure of digestibility. For wheat, barley, and rye, the glycemic index of the breads containing whole kernels was considerably lower than that of the breads made completely from barley flour. Interestingly, the glycemic index of the breads did not differ for oats, possibly because the boiling pretreatment caused a disruption of the oat groats but not of the rye, wheat, or barley kernels. In vitro results correlated closely with the glycemic index data.

Starch granules are surrounded by other plant materials (e.g. cell wall components) that can inhibit access of amylases to the granule. In some processed foods, protein may encapsulate the starch granules. Before digestion of the starch can occur, this protein must be digested by proteases. Examination of in vitro digestion of starch in red kidney beans (*Phaseolus vulgaris*) revealed that amylolysis was enhanced by wet homogenization and pepsin pretreatment (94). These findings indicate that disruption of cell walls is a prerequisite for efficient digestion.

Under some processing conditions, the long α-glucan chains can form inclusion complexes with fatty acids (36). These complexes can affect digestibility. In vitro susceptibility to porcine α-amylase of amylose-lipid complexes is reduced compared with free amylose. However, complete digestion of amylose can still be achieved if α-amylase levels are increased. Studies of digestion of the amylose-lipid complex in rats showed that although small intestinal breakdown was somewhat retarded, it was nevertheless complete.

STARCH STRUCTURE Examination of starch granules of different sizes from cassava and corn suggests that the smaller the granule, the greater the extent of in vitro digestion by bacterial α-amylases and fungal amyloglucosidase (26). In these studies, the content of amylose was greater in the smaller starch granules, but degradation of the amylopectin portion was favored. Higher levels of amylose in starch granules per se do not appear to affect digestibility. Fujita et al (28) examined in vitro digestion of high-amylose starch granules of maize and found that one variety (*su2*) was more susceptible than normal maize, whereas another (*ae*) was less susceptible. These differences were also

noted when the starches were fed to rats, although the total digestibility of the starches was greater in vivo than in vitro. The digestibility of starches from some plant species is much less than that of others. Raw potato and field bean starches are poorly digested in vitro compared with most cereals. Rice and tapioca starches (12) are digested particularly well in vitro and also have lower levels of amylose. Potato starch granules exhibit a predominantly B-type crystalline structure and, when isolated, are less susceptible to α-amylase hydrolysis than are A-type structures (98). However, these differences may be the result of surface area. Potato starch granules are very large and therefore have a low surface area relative to volume compared with B-type spherulites, which are rapidly fragmented by amylases, a process that increases the area exposed to attacking enzymes.

Extrinsic Factors and Digestibility

PROCESSING Starch in processed foods may be essentially unchanged, partially or wholly gelatinized, or partially retrograded. Raw starches are highly resistant to enzymic hydrolysis compared with gelatinized starches. The extent of hydrolysis of pea, maize, wheat, and potato starches is enhanced greatly by gelatinization, particularly at low levels of hydrolytic enzyme (67). The conditions under which the gels were prepared in this study are not representative of those of food processing. Nevertheless, similar results were obtained when starches were examined in cooked foods. Boiling or pressure cooking of presoaked peas (*P. sativum*) increased in vitro starch digestibility by 40–50%, apparently as a result of gelatinization (3). Colonna et al (9) obtained similar data on the effects of cooking on in vitro enzymic susceptibility of starch from pasta. They also noted that protein encapsulation inhibited starch digestion. The crucial role of starch granule disorder has been illustrated in a study in which barley starch granules were subjected to different temperatures (50, 55, and 60° C for 3 h) (48). Gelatinization of the starch granules occurred at 60° C, with a concomitant large increase in α-amylase digestibility. The presence of moisture during the heating process is critical. Dry heating (at 50, 80, and 90° C) of starch in spaghetti has very little effect on in vitro α-amylase digestibility (6).

Other factors also can influence starch retrogradation. In the sweet potato, for example, soluble sugars (sucrose, glucose and fructose) inhibit starch retrogradation (46). Additionally, soluble arabinoxylan—an endosperm cell wall constituent of cereals—inhibits retrogradation (34). Complexing of α-glucan chains with lipids inhibits retrogradation as well (83) but may reduce starch digestibility because complexes are degraded more slowly than free amylose (36).

Determination of Resistant Starch in vivo

One way to assay physiologically resistant starch is to isolate and examine starch from the lower ileum in humans with ileostomy. Such a study of the structure of physiologically resistant starch was reported recently (21). Retrograded maize starch, maize starch complexed with monoglycerides, potato flakes, and bean flakes were fed to human volunteers (healthy and ileostomates). Terminal ileal samples were recovered by intubation or from ileostomy bags. Analysis of starch recovered from the terminal ileum revealed that in all cases, the starch consisted of three populations of α-glucans—those of low (DP 1–5), intermediate (DP 13–38), and high (DP < 100) molecular weight. The first fraction was completely water soluble, as were parts of the other two fractions. DSC and birefringence analysis revealed that a portion of the starch in the ileal samples was highly crystalline, with a B-type pattern, whereas the other portion was amorphous. The resistance of the latter portion was partly due to its encapsulation by other components, such as cell walls. Further studies on in vivo resistant starch from the intubated subjects indicated that their physicochemical properties differed somewhat from those of chemically resistant starch fractions isolated from the foods (20). Thus starch fractions that appear digestible by in vitro techniques reached the lower ileum in these subjects. These studies highlight the fact that several factors, such as transit time, physical inaccessibility, amylase concentration, and the presence of other food components, influence the enzymic hydrolysis of starch and contribute to the amount of starch that reaches the terminal ileum.

Manipulating the Levels of Physiologically Resistant Starch

Schweizer et al (74) analyzed foods (instant bean flakes or potato flakes) for chemically resistant starch as defined by Englyst et al (17). When these foods were consumed by human ileostomates as part of a mixed diet, all of the resistant starch was recovered in the ileal effluent. Faulks et al (22) prepared chemically resistant starch from maize starch and pea starch and fed it to rats. They demonstrated that although the pea resistant starch appeared to be almost completely indigestible in the small intestine, ~ 50% of the chemically resistant maize starch was digested. In 1992, Tovar et al (93) examined the digestibility of legume starches (beans and lentils) and reported that at least 90% of fed retrograded starch was recovered from the feces of rats. This starch must have survived passage through the small intestine, but it was also resistant to microbial degradation in the large bowel. Starches with this level of resistance are of limited nutritional value compared with starches that survive transit through the small intestine but may be fermented in the large bowel. Indeed,

it is possible that such starches could be considered analogs of cellulose rather than as fermentable polysaccharides.

Addition of indigestible nonstarch polysaccharides may also inhibit digestion of starch. This inhibition has been noted in broiler chickens in which cell wall polysaccharides may cause considerable production problems (8). Fenugreek gum has been shown to inhibit in vivo starch digestion in rats and limit glucose absorption in vitro in everted sac preparations (53). In the pig, which is arguably a better model for the human than the more commonly used rat (32), addition of guar gum (6%) to a semipurified diet reduced ileal digestibility of starch to below 80% compared with control diets in which the starch was almost 100% digestible (49). Steinhart et al (80) showed that in human ileostomates fed various foods, the recovery of available carbohydrate (starch by difference) in the ileostomy effluent correlated positively with fiber intake. Stepwise regression revealed that fiber intake was the principal factor in determining the amount of available carbohydrate excreted. The mechanism by which fiber inhibits starch digestion remains uncertain, but it may relate to the viscosity of the digesta or to a direct inhibition of α-amylase enzymes.

Nutritionists take many factors other than retrogradation into account. These are frequently overlooked in in vitro determinations of resistant starch in which foods have been incubated with amylases and glucose release measured. Such procedures are of doubtful relevance to the human gastrointestinal system, and efforts have been made to duplicate the digestive process more closely by incorporating chewing by humans (62). As expected, incompletely masticated foods released less glucose. However, response varied with more resistant starch in poorly chewed baked beans and whole rice, but not in ground rice, pearl barley, and cornflakes. Retrograded or chemically resistant starch must therefore be regarded as the lowest content of resistant starch in a food. Moreover, physiologically resistant starch may increase considerably the total amount of resistant starch.

PHYSIOLOGICAL CONSEQUENCES OF RESISTANT STARCH

Resistant Starch as Malabsorbed Carbohydrate

Incomplete digestion of carbohydrates in the small intestine can result in a malabsorption of simple carbohydrates, which can pose serious health problems with simple carbohydrates. Lactose intolerance arises from a lack of the appropriate disaccharidase. The symptoms are the same as for the other poorly absorbed simple sugars when ingested in quantity—diarrhea, abdominal pain, and cramps. These reactions result from the accumulation of sugars in the large bowel in quantities too large for fermentation by the microflora, which leads

to osmotic diarrhea (37). This mechanism of action is the same as that for certain laxative products, such as lactulose.

Similar problems have occurred with dietary water-soluble nonstarch polysaccharides, especially in experimental animals. Inclusion of isolates such as gum arabic (90) as single fiber sources at high levels in purified diets leads to fluid accumulation in the cecum of rats and to diarrhea. The intake of nonstarch polysaccharides under these conditions is much greater pro rata than that of humans consuming such products. However, abdominal discomfort has been recorded in humans consuming guar gum (85), presumably as a result of fluid retention and fermentation. The response to resistant starch seems to depend greatly on the food in which it is found. For example, the consumption of cornflakes (which provide ~ 7.5 g of resistant starch per person per day) caused no overt discomfort (87). Legumes are considered good sources of resistant starch because of the presence of amylase inhibitors and other factors that lower starch digestibility. Consumption of red kidney beans by human volunteers was reported to stimulate intestinal gas production owing to colonic fermentation (24). In this study, the subjects consumed 100 g (dry weight) of beans per day and an average of 42 g of fiber per person per day. Surprisingly, no differences in fecal excretion were observed, and the subjects reported no other symptoms. In general, foods containing resistant starch do not seem to pose any of the problems associated with malabsorption of other carbohydrates.

Resistant Starch and the Glycemic Index

Clearly, the passage of undigested starch into the colon can limit the amount of glucose that can be absorbed in the small intestine. Foods high in resistant starch should yield lower glycemic index values than similar products, but the data are contradictory. Although some studies have demonstrated a good correlation between in vitro hydrolysis rates and in vivo responses, others have not. For example, Brand Miller and colleagues (2) carried out studies on white and brown rice from a cultivar (*Doongara*) high in amylose (28%). They found a lower glycemic index than in comparable products from other cultivars (*Calrose* and *Pelde,* each of which contains 20% amylose). However, these differences were not significant. For example, in eight subjects consuming rice containing 28% amylose, the mean glycemic index was 64, whereas rice with 0–2% amylose yielded a glycemic index of 88.

Truswell (95) reviewed the relationships between resistant starch and glycemic index and noted that high glycemic index values had been reported in humans consuming potatoes and cornflakes—foods that can contain significant amounts of retrograded starch. Only certain foods, such as legumes and pasta, yielded low glycemic index values commensurate with the proportion of enzyme-resistant starch in the test meal. Truswell listed several methodological factors that could potentially contribute to these inconsistencies, including the

quantity of the food that was fed and day-to-day variations within the same individual. Equally important are the in vitro conditions used to assay starch hydrolysis. In such determinations, resistant starch may equate with the slowly digested starch rather than with the resistant starch defined by Englyst et al (18). The former may appear resistant in vitro but is digested completely in the small intestine if transit is sufficiently slow.

By integrating measurements of dietary resistant starch, ileal starch excretion, and glycemic response, Schweizer and colleagues (74) determined that when the amount of resistant starch in foods falls below a certain level, it cannot be detected. They compared potato flakes (low resistant starch) and bean flakes (high resistant starch) and found that only 16% more starch was digested and absorbed from test meals of the former, whereas glycemic responses were almost twice as high.

Starch and the Large Bowel

Although in vitro studies support the concept of starch malabsorption, evidence that starch enters the large bowel and information on the quantities involved have been more difficult to obtain in vivo. The excretion of starch (and other nutrients) in humans with ileostomy is highly suggestive. These experiments also support the view that the quantities involved may be significant. For example, when compared with a basal diet, an additional 13.7 g of starch (expressed as available carbohydrate) was excreted in ileostomates consuming red kidney beans, whereas only an additional 1.6 g was excreted in the same patients when they were fed white bread (57). In both cases, starch consumption was similar. However, ileostomates are an atypical population, having undergone defunctioning bowel surgery for various reasons. The appearance of starch and other nutrients in the ileal effluent of such patients does not necessarily signify that starch enters the large bowel of intact humans. Support for the entry of starch into the colon comes from several lines of research.

THE CARBOHYDRATE GAP Feeding of foods containing nonstarch polysaccharides (fiber) leads to increased fecal bulk in humans and animals. Products such as wheat bran increase stool mass in humans in a dose-dependent manner (42). However, the increase in fecal bulk in humans eating mixed diets is considerably higher than predicted from their nonstarch polysaccharide content—the "carbohydrate gap" (81). Starch is thought to fill this gap and contribute to the greater fecal bulk through bacterial proliferation. Cummings & Macfarlane calculated that in the British diet, ~ 8–40 g of enzyme-resistant starch could enter the bowel daily, compared with 8–18 g of nonstarch polysaccharides (15). In Australia, which has a higher average fiber consumption (1), the contribution of starch may be even greater.

BREATH HYDROGEN EVOLUTION The response of breath hydrogen in human volunteers fed test meals provides support for a significant role of starch in colonic metabolism. The main products of colonic carbohydrate fermentation are short-chain fatty acids, increased bacterial mass, and the gases CO_2, CH_4, and H_2 (15). The latter gas can be detected in the breath of humans. Comparisons between products low and high in resistant starch (14, 25, 87) have revealed greater evolution when starch malabsorption would have been expected. However, this method cannot discriminate between resistant starch, nonstarch polysaccharides, and other substrates that generate H_2. Evidence of H_2 evolution from the stomach (69), which is consistent with bacterial activity may confound this hypothesis, but it seems rather insignificant compared with the stronger evolution of H_2 from the colon.

ANIMAL STUDIES Feeding of raw (56) starch or retrograded amylose (prepared by gelatinization of high-amylose cornstarch) (22) leads to increased cecal digesta mass. In animals fed raw or retrograded starch, cecal carbohydrate and short-chain fatty acid concentrations are also increased. This outcome is consistent with enhanced fermentation (22, 31, 50). Furthermore, studies in pigs with cecal fistulae reveal that legumes increase large-bowel digesta and short-chain fatty acids (23).

COMPLEX CARBOHYDRATES AND LARGE-BOWEL FERMENTATION

Large-bowel fermentation is stimulated by the presence of complex carbohydrates, including starch and nonstarch polysaccharides. The main short-chain fatty acids produced are the same as those in the rumen, i.e. acetate, propionate, and butyrate, which contribute over 90% of the total fatty acids in the large bowel of humans, rats, and pigs as well as in ruminal fluid. However, some differences among species and with individuals exist. For example, some humans have a so-called acetogenic pathway that can change the proportions of the gases evolved and of the acids produced during carbohydrate fermentation (47). Activity of this pathway may modify the products of fermentation in certain individuals.

Metabolism of the Major Short-Chain Fatty Acids

Many of the important effects of complex carbohydrates in the large bowel probably can be attributed to their metabolism to short-chain fatty acids. In this regard, resistant starch does not differ from other carbohydrates, including many nonstarch polysaccharides. Studies in experimental animals have revealed that the feeding of fermentable carbohydrates increases the concentra-

tions of short-chain fatty acids in large-bowel contents of animals such as rats (e.g. 90) and pigs (e.g. 45).

Short-chain fatty acids are absorbed from the large bowel, enter into the portal circulation, and are transported to the liver. Their concentrations have been measured in portal venous plasma blood of rats and pig and generally fall in the range of 0.4–2.0 mmol\liter (31, 66, 89). In both species, concentrations vary with experimental conditions and are higher with diets that contain fermentable polysaccharides. Concentrations have also been measured in humans postmortem and following trauma. Although these concentrations are considerably lower, they are still within the range found in animals (15). It is very difficult (ethically and practically) to obtain samples of portal venous blood or of large-bowel digesta from humans under controlled conditions. Therefore, the lower values found in humans probably reflect the unusual conditions under which the samples were taken rather than any intrinsic species difference.

Short-chain fatty acid concentrations in mixed venous and arterial blood are low owing to the uptake of short-chain fatty acids by the liver and other viscera, and usually only acetate is present in significant quantity. Rerat and colleagues have examined simultaneously the concentrations of short-chain fatty acids in portal venous and arterial blood of pigs. They found significant variations in the concentrations of propionate and butyrate in the former vessel, with little or none of either acid appearing in arterial blood (66). Of the individual acids, butyrate is taken up by the cells of the colon wall so that proportionately less appears in the portal vein. However, in both liver (79) and heart (92), acetate uptake is a net process, i.e. uptake occurs above a certain concentration, whereas release occurs below that concentration. Acetate release by the liver increases following ethanol ingestion, which accounts for the high blood acetate concentrations in problem drinkers (99). Hepatic release may also increase in diabetes (86). In liver, acetate is thought to compete with lactate for oxidation and for de novo fatty acid synthesis (79). The same probably holds true for other tissues as well, e.g. lung and adipose tissue.

Propionate is an acid of great metabolic importance to obligate herbivores such as ruminants, which rely heavily on gluconeogenesis to meet their glucose requirements (19). Of the short-chain fatty acids, propionate is the only one that can effect net conversion to glucose. Although propionate is clearly important for herbivores, it is difficult to determine whether it plays a similar role in omnivores (4). Propionate may inhibit the synthesis of fatty acids in the liver (probably through competition with lactate), thereby lowering the rates of triacylglycerol secretion (63). Nevertheless, this inhibition of fatty acid synthesis has not been consistent (38), and its occurrence remains to be established unequivocally. Propionate may be involved in the control of hepatic cholesterol synthesis. It has been proposed that complex carbohydrates that lower plasma cholesterol concentrations do so through inhibition of hepatic

cholesterogenesis via propionate formed through large-bowel fermentation (7). However, experimental evidence does not support this hypothesis (38, 63), and this process appears unlikely to occur in vivo. Propionate may also affect large-bowel blood circulation. Mortensen et al demonstrated in vitro that propionate dilates the resistance vessels of the large-bowel circulation (61). This dilation would lower resistance to flow and may explain the higher blood flow observed in animals fed fiber. Propionate has also been shown to stimulate large-bowel epithelial proliferation, which may help maintain epithelial integrity (71). Furthermore, Yajima (101) reported that propionate stimulates muscular contractile activity in isolated rat colon. This activity could assist in fecal expulsion in vivo.

Butyrate has attracted much attention. It appears to be a preferred substrate for normal colonocytes (68) and assists in the maintenance of colonic integrity. Butyrate inhibits the growth of transformed cells in vitro (5) and promotes a normal cell phenotype by enhancing the stabilization of DNA and repair of damage (96). Butyrate is thought to play a key role in the prevention of colonic cancer. In fact, experimental studies suggest that foods that promote butyrate formation in the hind gut (e.g. wheat bran) also lower tumor mass in experimental models of cancer (58). Direct infusion of butyrate into the colon leads to remission of ulcerative colitis (73).

In addition to producing the aforementioned specific effects, short-chain fatty acids promote colonic health by inhibiting the growth of pathogens, enhancing fluid and electrolyte absorption, and moderately lowering pH (91).

Short-Chain Fatty Acids as Mediators of the Effects of Resistant Starch

Water-soluble nonstarch polysaccharides are fermented extensively in the large bowel, and some of their effects in this viscus may be due to short-chain fatty acids and not polysaccharides per se (88). This is a reasonable argument that could apply also to resistant starch. Although resistant starch has been proposed to provide specific benefits, the evidence supporting such an association is not yet abundant. Cummings & Macfarlane (15) concluded from in vitro studies with human fecal inocula that, of the polysaccharides studied, starch was the best substrate for butyrate production. Given the putative role of butyrate in colo-rectal carcinogenesis, this observation is of considerable significance. The findings of Sheppach et al in humans consuming starch with the α-amylase inhibitor (arcabose) lend further support to the hypothesis that starch promotes butyrate production in vivo (72). In this study, feeding starch with the inhibitor promoted starch and butyrate excretion. However, evidence of a major role for resistant starch as a specific butyrate precursor is not yet adequate to make an accurate assessment. Fleming et al (24) demonstrated little or no change in fecal short-chain fatty acid excretion in humans under conditions in which

fermentation in the proximal colon appeared to be enhanced by feeding legumes. In vitro fermentation studies of ileal effluents (from humans fed a variety of foods) incubated with fecal microflora revealed no difference between total short-chain fatty acid production and starch excretion (57). Butyrate production was also unrelated to starch excretion.

The discrepancies in the fermentation data may reflect differences in the use of gut contents as opposed to purified polysaccharides or they may be a result of the requirements for other factors in the culture. Studies in rats fed resistant starch show that the flux of ammonia in the cecum was increased (65). This increase may be due to the demands of the microflora for nitrogen for growth. If nitrogen were inadequate in vitro, the data could well be compromised. Discrepancies in the effects of starch on the large bowel in vivo may be related to another complex issue—digesta passage along the colon.

Distribution of Short-Chain Fatty Acids in the Colon

Indirect methods (e.g. breath H_2) for assessing fermentation are limited, and short-chain fatty acids in peripheral venous blood are not representative of events in the gastrointestinal tract. Venous blood acetate has been reported to rise following the ingestion of fermentable carbohydrate, including starch (14), but such data offer few insights into the changes in other short-chain fatty acids. Data from human trauma victims (15) and from surgical patients (60) show that short-chain fatty acid concentrations fall from the proximal toward the distal colon (Table 2). This finding is consistent with the concept that short-chain fatty acid production predominates in the proximal hind gut and is supported by a study in which fecal inocula were taken from sudden-death victims and incubated in a batch culture system (52). Short-chain fatty acid production was up to eightfold higher with inocula from the proximal than from the sigmoid colon. No change occurred in the proportions of the major acids formed with sampling site, but the data suggest that caution should be exercised in extrapolating results obtained with fecal inocula to short-chain fatty acid production in the proximal colon.

The distribution of short-chain fatty acids in the porcine large bowel approximates that in humans (Table 2). Studies in pigs have revealed that short-chain fatty acids are raised by feeding foods that contain nonstarch polysaccharides. However, in animals fed certain foods cooked for human consumption, e.g. brown rice (54) and navy beans (89), total short-chain fatty acids and digesta mass were increased disproportionately. This discrepancy was ascribed to resistant starch. Approximately 15% of total starch was enzyme resistant in navy beans (GB Storer & DL Topping, unpublished observations), but less than 1% was resistant in rice (55), which suggests that other factors (e.g. lipid) may have retarded small intestinal digestion.

Of particular interest is the finding that in animals fed navy beans, short-

Table 2 Concentrations of short-chain fatty acids in human colon and pools of short-chain fatty acids in porcine colon

Group	Proximal colon	Median colon	Distal colon
	(mmol/kg of contents)		
Humans[1]			
Acetate	63	58	50
Propionate	27	23	20
Butyrate	25	24	18
	Pools (mmol)		
Pigs			
Navy beans[2]			
Acetate	13.9	3.2	0.8
Propionate	6.1	1.5	0.4
Butyrate	1.4	0.5	0.1
Brown rice[3]			
Acetate	10.2	5.8	1.5
Propionate	4.3	2.7	1.2
Butyrate	1.5	1.2	0.5

[1] From Ref. 65.
[2] Modified from Ref. 77.
[3] Modified from Ref. 101.

chain fatty acids (acetate and propionate) were higher in the proximal colon. These differences were not maintained in the distal colon. The data correspond with those obtained from humans in whom resistant starch did not induce any apparent change in colon function. In contrast, significantly more butyrate was found in the distal colon of pigs fed rice. Japanese subjects who habitually consume rice exhibit relatively high fecal butyrate excretion (84). These data suggest that starches from various plant foods may differ markedly in their fermentative characteristics.

FUTURE DIRECTIONS

Much is known about the factors that control starch retrogradation and the interactions between starches and other components in processed foods. However, their implications for starch digestibility in vivo are poorly defined, and the impact of malabsorbed starch on the colon is understood even less. This lack of knowledge partially reflects the difficulties inherent in studying human large-bowel physiology. Indirect methods of assessing fermentation provide little indication of the changes that occur in response to the entry of starch (or other food components) into the colon. Animal and other models are clearly

necessary, but they can be limited as well. The rat may be particularly unsuitable because it is a fecal refector. Abolition of coprophagy changes large-bowel short-chain fatty acids in ways that are not always predictable (41). Relating the data to humans is therefore problematic.

One may be tempted to treat resistant starch as a special case, particularly in view of the suggestion that its consumption may favor colonic bacterial butyrate production. Experimental evidence offers promise but is not yet conclusive and does not take into account the potential of other short-chain fatty acids to maintain colonic integrity. Other cereal products (e.g. those from wheat) also increase large-bowel short-chain fatty acids (including butyrate) in animal experiments.

With respect to fecal bulking, the contribution of starch may be much greater than that of nonstarch polysaccharides simply because more starch is eaten than other carbohydrates. This suggestion is of considerable importance because fecal bulk appears to be negatively related to colon cancer risk (13). However, the concept of starch as the primary contributor overlooks the fact that many cereal products are also good fecal bulking agents. Thus resistant starch should probably be considered as a component of dietary fiber and not a substitute.

At present, it seems unlikely that resistant starch has a substantial impact on plasma cholesterol. Its most important benefits seem to lie in the large bowel (as do those of fiber). In this regard, resistant starch offers a major advantage because it can be manipulated technologically to alter the apparent fiber content of foods without greatly changing their organoleptic properties. The consumer in particular may benefit from the availability of products high in resistant starch that do not differ radically from conventional ones. The imperative is to define more precisely the links between the physiological actions of resistant starches on the one hand and their chemical and physical properties on the other.

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