Digestion of Carbohydrate from White Beans (Phaseolus vulgaris L.) in Healthy Humans

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ABSTRACT Resistant starch (RS) is thought to be present in large amounts in legume seeds; however, it has never been quantified in healthy humans. RS from cooked (atmospheric pressure) white beans was quantified in humans and pigs, and characterized to explain its low digestibility. Six human volunteers were intubated to collect ileal digesta after an experimental meal composed of orange juice, butter and 167 g beans (dry matter basis). The reliability of the intubation method was examined in a pig study in which it was compared with another collection method, ileal cannulation. Chemical analyses, microscopy and size exclusion chromatography were performed on human and pig digesta. The pig study showed that the intubation method may underestimate the quantity of RS. However, no chemical/physical difference was observed between the RS collected by the two techniques. In the human study, 16.5 ± 1.3% (11.3 g) of the ingested starch was recovered as RS. The microscopy of the digesta showed that part of the RS was enclosed in the cell walls. Although the RS was composed mainly of α-glucan molecules with a degree of polymerization (DP) 40 to 60, oligosaccharides and large molecules of DP > 400 were also present. Retrogradation was not found to be the main factor responsible for starch malabsorption. We conclude that white beans may contain a large amount of RS formed mainly by partially degraded molecules protected by the cell walls during their transit through the gut. J. Nutr. 128: 977–985, 1998.

KEY WORDS: • beans • resistant starch • fibers • digestion • humans • pigs

Starch is one of the major components of human and animal diets, and originates mainly from cereals and legumes. Over the last 15 years, it has been shown that a small proportion of the starch present in some foods can escape hydrolysis by digestive enzymes, both in vitro and in vivo. “Resistant starch” (RS) was defined in 1992 by EURESTA (European Research Project on resistant starch) (Astrup 1992) as “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy humans.” It is estimated that 4–5 g of resistant starch is consumed per day in Western diets (Dysseler and Hoffen 1994), although Cummings and MacFarlane (1992) calculated a quantity of 8–40 g/day, which is similar to the amount of non-starch polysaccharides ingested daily (8–18 g). RS is fermented by bacteria in the large intestine to produce short-chain fatty acids, with a high proportion of butyrate (Cummings and MacFarlane 1992, Englyst and Cummings 1987, Scheppach et al. 1988). Foods that promote the production of butyrate are associated with lower risks of bowel cancer (MacIntyre et al. 1993). Whether the consumption of starchy food rich in RS is beneficial for health remains controversial. The low postprandial hyperinsulinemia/hyperglycemia associated with starchy foods rich in RS can influence the regulation of glucose metabolism (Lehrer-Metzger et al. 1996, Raben et al. 1994). It has also been found that RS may lower the level of plasma cholesterol (Behall et al. 1989), which might be a consequence of hepatic metabolism regulation (Sacquet et al. 1983). RS also increases fecal bulking and lowers fecal pH (Philipp et al. 1995), factors that are usually considered as markers of healthy colonic mucosa (Cummings et al. 1992).

The potential effects of resistant starch on health are highly related to its fermentation pattern. Not all of the RS is fermented to the same extent, or at the same rate, or even to the same metabolites (Edwards et al. 1996, MacBurney et al. 1990, Nordgaard et al. 1995). The physicochemical properties of RS may be major variables in determining the fermentation pattern. The main classification of RS has been proposed by Englyst et al. (1986); it is based on the nature of the starch and on its environment in the food. RS1 correspond to physically inaccessible starches, entrapped in a cellular matrix, as in legume seeds (Tovar et al. 1992, Würsch et al. 1992). RS2 are native uncooked granules of starch, such as raw potato or banana starches, whose crystallinity makes them less susceptible to hydrolysis (Englyst et al. 1987, Faisant et al. 1995). RS3 are retrograded starches, which may be formed in cooked foods...
that are kept at low or room temperature. The consequent reassociation of amylose leads to a semicrystalline structure that is resistant to hydrolysis, both in vitro (Colonna et al. 1992) and in vivo (Englyst and Cummings 1987, Molis et al. 1992).

Legumes, which are rich in starch and fibers, are of particular interest because they often contain RS1, but may also contain RS2 and RS3 (after cooking). Relatively few studies have tried to quantify the digestibility of starch from legumes in the small intestine of humans (Botham et al. 1995, Schweizer et al. 1990, Wolever et al. 1986); no studies have used the intubation method, which, in reality, is the only technique available with which to obtain direct sampling of the digestive contents from healthy subjects. However, the reliability of this technique has not yet been proven for the sampling of large particles.

Therefore, the objectives of this study were to quantify the amount of starch resistant to digestion in the small intestine of healthy humans, in home-cooked white beans (Phaseolus vulgaris sp.), to determine the factors responsible for the low extent of digestion of starch and to check the reliability of the results obtained by the intubation method in humans by performing a dual experiment on pigs with both the intubation and cannulation methods.

SUBJECTS AND METHODS

Substrates. Dry white beans (Phaseolus vulgaris L., haricots lingots, S. C. GaleC) were used in this study.

Experimental meal. Beans (167 g) were soaked for 4 h after which the soaking water was discarded. The beans were washed with water and then cooked for 1.75 h in boiling water. This cooking time was chosen because it was the minimum time required to render the beans palatable. They were cooled and kept overnight at −20°C before being reheated. Beans prepared “as eaten” were analyzed for their content of total starch, in vitro resistant starch, soluble and insoluble fibers, neutral sugars, uronic acids and proteins.

The experimental meal (2262 kJ) for both humans and pigs was composed of the cooked beans mixed with 17 g of butter. Subjects and animals also drank 100 mL of orange juice. Human volunteers were allowed to drink coffee, tea or water with no more than 10 g of sugar.

First experiment: human study

Subjects. Eight volunteers took part in this study. Results obtained from only six of the volunteers (3 men, 3 women) were used for several reasons. One subject took 30 min more than the others to eat only two thirds of the meal. The data from a second subject could not be used because introduction of the flow marker, polyethylene glycol (PEG), into the ileum was unintentionally interrupted for 2 h during the study. All subjects were nonobese (body mass index (BMI), 22.3 ± 0.9 kg/m² for women and men, respectively) and in good health. They had no history of gastrointestinal diseases and had not received any treatments including antibiotics or laxatives during the last 2 mo before the study. The protocol was divided into two periods.

First period: collection of the effluents at the end of the small intestine. The volunteers were nasally intubated with a polyvinyl catheter to ensure collection of the digesta passing through the ileum. The day before the study, the subjects received polysaccharide-free meals. On the morning of the study, they ingested the experimental meal. The collection of the digestive content was started 30 min before the subjects began the breakfast, and was performed continuously for the next 14 h. Samples were collected every 30 min, frozen in liquid nitrogen and stored at −70°C until analysis. An aliquot was used to measure the dilution of the infused PEG (Faisant et al. 1995, Flourie et al. 1988, Stephen et al. 1983). PEG is used as a dilution marker to determine the flow of the digestive content at the end of the ileum. It is perfused 25 cm above the sampling site to allow an optimal homogenization and minimize the difference in flow rate between the solid phase and PEG, which is indeed a marker of the liquid phase. The concentration of PEG was used to calculate the volume of intestinal content flowing through the terminal ileum during each 30-min period.

For each subject, two aliquots, representing 5% of the dry matter of each 30-min sample, were used to make a total pool and a partial timed pool. The total pool was comprised of aliquots from all of the samples collected during the 14 h. The partial timed pools were comprised of fractions collected over a 2- to 3-h period: 0–2.5, 2.5–5.5, 5.5–8.5, 8.5–11 and 11–14 h after the meal. These pools provided sufficient amounts of dry matter to enable physical and chemical analyses of the samples.

Total α-glucans (RS) and PEG were quantified in each 30-min sample. Several samples were taken for light microscopy examination. Further chemical and physical characterizations of the ileal samples were performed on three of the six subjects. They were selected according to the efficiency of the collection so that a sufficient amount was available. Moreover, the BMI of these subjects (2 men and one woman) was not different from that of the whole group (22.95 ± 1.6 vs. 22.90 ± 0.8 kg/m²). For these subjects, total α-glucans, oligosaccharides (1–10 glucose units), neutral sugars, uronic acids and proteins were analyzed in pooled samples. The fraction of α-glucans still potentially digestible was quantified in vitro. The products of starch degradation were analyzed by size exclusion chromatography.

Second period: stool collection. Over the period of stool collection (8 d), the digestibility of the starch ingested was determined after the passage through the large intestine. Subjects ingested the same quantity of cooked beans daily (equivalent to 167 g dry weight of beans). They were asked not to eat any other starchy food and had to record each of their meals. The consumption of dietary fibers was not limited, but fiber consumption for each subject was calculated from the food records. After a period of adaptation of 4 d, stools were collected daily from each subject, weighed and stored at −20°C. Total digestibility was calculated over the last 4 d as follows: (100 [intake − fecal excretion]/ intake).

Total α-glucans were analyzed in the stool for all the subjects. Uronic acids, neutral sugars and proteins were analyzed for three subjects.

Ethical considerations. All of the subjects gave a written informed consent to the protocol, which was approved by the local human ethics committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Région Pays de la Loire, Nantes, France).

Second experiment: pig study

Animals. Three female Large White pigs weighting 30–35 kg at the time of the experiment were used for the study. The animals were purchased at INRA (Saint-Gilles, France). They were fitted with simple T-ileal cannulas, as described by van Leuwen et al. (1991), and with an intestinal catheter. Unlike the study in humans, the double-lumen catheter was surgically introduced into the intestine about 40 cm before the ileo-cecal junction. Animal treatment was in accordance with French legislation.

Collection of the effluents at the end of the small intestine. Pigs were fed twice a day. The day before each study, they received polysaccharide-free meals to prevent the collection of an undigested fraction during the experiment. The morning of the study, the pigs ate the experimental diet described above.

Intubation technique. Five trials were performed using two of the pigs. Samples were collected continuously during a 14-h period. PEG was used as the flow marker as described in the human study.

Cannulation method. Seven trials were performed with the three pigs. Cannulas were rinsed with distilled water before the study to prevent bacterial fermentation. The day of the study, an occlusive rubber balloon was inflated in the intestine just below the cannula to provide a total collection of the digesta. A solution of PEG was infused by the catheter continuously during the study, as described above. The reliability of the collection was checked by the recovery of PEG in the samples. The collection of digesta lasted 14.5 h, beginning 30 min before the meal. Bags were changed every 30 min, and...
Digesta were immediately frozen in liquid nitrogen, stored at -70°C and later freeze-dried. For each pig, aliquots of samples were pooled together, as indicated for the human study.

Analyses of total α-glucans and PEG were performed on each sample. Pooled samples were analyzed for the determination of total α-glucans (RS), oligosaccharides (1–10 glucose units), neutral sugars, uronic acids and proteins.

Analysis of beans, ileal contents and stool

Chemical analysis. Starch, as total α-glucans, was analyzed by the method described by Faisant et al. (1995). Resistant starch was estimated in vitro by the method of Englyst et al. (1986). Soluble and insoluble fibers were analyzed by the method of Prosky et al. (1988). The method of Hoepler et al. (1989) was used to analyze the neutral sugars; uronic acids were dosed by the method of Blumenkranztz and Asboe-Hansen (1973), automated by Thibault (1979). Proteins were quantified according to the Kjeldahl method, with 5.7 as a conversion factor. Oligosaccharides (1 to 10 glucose units) from starch degradation were extracted from pooled samples and analyzed. After suspension in water (200 mg in 10 mL), the samples were centrifuged at 10,000 × g for 15 min. The supernatant was evaporated and the residue solubilized in 5 mL of 80% ethanol (v/v) in water. The solution was again centrifuged (10,000 × g, 15 min) and the supernatant evaporated. The final residue was solubilized in pure water. Total oligosaccharides were hydrolyzed with a thermostable amyloglucosidase (cat. no. 1332, 2217 nkat/ml glucose equivalent, Merck-Clévenot S. A., Nogent-sur-Marne, France) for 90 min at 60°C. Free glucose was analyzed by the enzymatic NADP-ADP/hexokinase/glucose-6-phosphate dehydrogenase system (cat. no. 127825, Boehringer Mannheim, Meylan, France). PEG (flow marker) was quantified in the samples of digesta by the turbidimetric method of Hyden (1955).

Physical characterization. Light microscopy was used to examine the structure of cotyledon cells in the soaked and cooked beans or in the samples of digesta. The technique of Ben-Hlich (1993) was used to visualize starch (stained purple), cell walls (stained orange) and intracellular proteic matrix (stained green). The samples were first hydrated, then frozen and cryosectioned (sections were 5–10 μm thick). Samples were then colored with “acridine orange” (1 g/L) for 5 min. After the samples were washed with water, acridine orange fixed on the preparation was precipitated by rouge Congo (1 g/L) for 5 min. Samples were washed again in water before being colored with fast-green (1 g/L) and 0.02 mol/L iodine for 2 min. They were rinsed with water before examination.

The crystallinity of starch in raw or cooked beans was examined using X-ray diffraction analysis. The patterns were obtained at 40 kV and 30 mA by using an INEL XRG 3000 generator (INEL, Artenay, France). The X-ray radiation was selected with a quartz monochromator and detected with a curve position sensitive detector (INEL CPS 120).

Size exclusion chromatography (SEC) was performed on a Superose 12TM column (Pharmacia Biotech, Uppsala, Sweden): 30 cm length, 1.0 mm diameter, eluent 0.1 mol/L KOH, 20 mL/h. Samples were first mixed under liquid nitrogen; then 25 mg starch equivalent/mL was solubilized for 24 h in 1 mol/L KOH under mixing, and diluted with water down to 0.1 mol/L KOH. After centrifugation (10,000 × g, 10 min), 100 mL of supernatant was injected. Fractions of 0.33 mL were collected from the column and neutralized with 0.33 mL of 0.1 mol/L HCl. Then 0.65 mL of citrate buffer (pH 4.8) and 0.25 mL of enzyme solution (amyloglucosidase thermostable) were added to the mixture and incubated at 60°C for 90 min. Glucose released in each fraction was measured by spectrophotometry at 620 nm, using the glucose oxidase (GOD)-peroxidase (POD)-2,2'-azino-bis(3-benthiazoline-6-sulfonate) (ABTS) reagent (100 mg GOD, type II Sigma [Saint-Quentin Fallavier, France], 3 mg POD, type I Sigma, 50 mg ABTS, Boehringer Mannheim, Germany). The column was calibrated for linear α-1,4-glucans, as described by Leloup (1989).

The calibration curve obtained for the human study was as follows: log DP = 0.6175 - 2.2400 Kα, where DP is the degree of polymerization and Kα (the apparent reaction order) is defined as follows: (Vc – Vo)/(Vt – Vo), where Vc is the elution volume of the sample, and Vo (exclusion volume) and Vt (total volume) are determined with amylose (from potato starch, prepared locally) and glucose, respectively (Merck-Clévenot cat. no. 1,08342). The different chromatograms showed peaks characterized by DP max (degree of polymerization at its maximum), DPn and DPw; DPn = E/Ci(DPi); DPw = (Σ Ci·DPi)/Σ Ci where Ci and DPi are the α-glucan concentration and the degree of polymerization of each fraction, respectively. In addition, various hydrolysates of beans were submitted to SEC to elucidate the origin of the α-glucans resistant to digestion as follows: retrogradation of starch (SEC 1), role of the cell walls (SEC 2), network protein (SEC 3) and/or the presence of highly branched α-glucans (SEC 4).

SEC 1 (Lintnerized sample). Beans prepared “as eaten” were hydrolyzed by 2.2 mol/L HCl, as described by Faisant et al. (1993), to isolate the fraction of starch that was the most resistant to digestion (i.e., the lintnerized starch). This fraction was then placed in a dry oven at 40°C overnight and then carefully ground before it was analyzed by size exclusion chromatography (SEC).

SEC 2 (Milled sample). Beans prepared “as eaten” were freeze-dried and milled under liquid nitrogen to disrupt the cell wall structures. They were then submitted to hydrolysis by α-amylase for 16 h according to the method of Champ (1992). The hydrolysate was kept for analysis by SEC.

SEC 3 (Deproteinized sample). Beans “as eaten” were freeze-dried and milled. Dry matter of the milled flour (1 g) was deproteinized with 5 mg thermostable protease (Sigma P-5380) for 30 min at 60°C in 50 mL of phosphate buffer (pH 7.5). The solution was then centrifuged (3000 × g, 10 min) and rinsed three times with 50 mL of water. The pellet was subsequently hydrolyzed by α-amylase as above and analyzed in SEC.

SEC 4 (Debranched sample). Beans “as eaten” were freeze-dried and milled. Powder, containing 100 mg of equivalent starch, was solubilized in 10 mL sodium acetate buffer (pH 5.2) and hydrolyzed by 120 μL of pullulanase (Sigma P-5420) and 200 mg of α-amylase (Sigma A-3176) for 16 h at 42°C (Englyst et al. 1982). This enzymatic treatment was performed to hydrolyze α-1,6 linkages of starch. The products of hydrolysis were removed by the addition of 40 mL of absolute ethanol and centrifugation (3000 × g, 10 min). The pellet obtained was rinsed twice with 10 mL of 80% ethanol, then dried before analysis.

Calculation and data analysis. With the intubation technique, starch malabsorbed by each of the subjects and pigs was calculated as follows: X(α-glucans [g/mL] × ileal flow rate [mL/min] × time [min]) / 100/amount of starch ingested [g]. Ileal flow rate = Calculated flow rate – flow rate of perfusate; Calculated flow rate = (PEG concentration in perfusate [g/mL]PEG concentration in ileal sample [g/mL]) × flow rate of perfusate [mL/min]. Values are expressed on a dry matter basis, as means ± SEM.

RESULTS

Analysis of the cooked beans. After cooking, the beans were composed (on a dry matter basis) of 286 g/kg protein, 304 g/kg total fiber, which was mostly insoluble (82%), and 410 ± 10 g/kg (n = 7) total α-glucans, including 29 g/kg free glucose. Resistant starch of the cooked beans was estimated in vitro to be 17.1 ± 1.4% (n = 6) of the total starch (7.0 ± 0.9% of the total dry matter).

As shown by light microscopy (Fig. 1A), cooking the seeds led to the separation of the cotyledon cells. The cotyledon cells were apparently not disrupted and contained starch granules, colored with iodine, surrounded by protein.

The retrogradation of starch after cooking was confirmed by X-ray diffraction analysis (see arrows showing the characteristic peaks in Figure 2); starch turned from a C pattern (Fig. 2A), characterizing most native legume starches, to a B pattern (Fig. 2B), which is believed to be due to a recrystallization of amylose after gelatinization and retrogradation (Colonna et al. 1992).
The non-starchy fibers were not totally recovered from the ileum. Although 61.0 ± 1.5% of uronic acids were recovered (3 subjects), the recovery of neutral sugars was 73.7 ± 7.3%. Xylose and arabinose are the major cell wall sugars in the hulls and cotyledons of the beans. Assuming that these sugars in the ileal digesta arise exclusively from the beans, the ratio of xylose to arabinose was used to detect selective recovery of either cotyledons or hulls found as a function of the collection time (Table 2). The ratio of xylose to arabinose in the pooled samples was very close to that found in the cotyledons of the beans (0.22).

Microscopic examination of the digesta. Samples collected from the ileum during the study showed a distinct change in the cellular structures in digesta with time. Some intact structures were observed at the first times of collection (3 h, 7 h after the meal), with starch enclosed in the cells (Fig. 1B). At the end of the experiment (11.5 h), only fragments of cell walls could be found. No free starch granules, outside the cellular matrix, were detected at any time.

Characterization of the resistant starch fraction. Size exclusion chromatography showed that the α-glucans recovered from the ileal effluents were composed of three fractions (Fig. 5). The first fraction (peak at $K_{av} = 0.0$) comprised large molecules having a DP > 400. They represented about 15% of the total α-glucans. The second fraction was the main one, and represented about 75% of the total α-glucans. Its maximum peak at $K_{av} = 0.39$, corresponds to a Dpn = 40. The Dpmax, Dpn and DPw

Digestibility of starch in the small intestine: human study

Digesta flow through the ileum. Real digesta flow was calculated for each 30-min period to examine the variations during the 14 h of the study. The liquid flow was very unstable after the meal, varying from 0.6 ± 0.1 to 2.3 ± 0.3 mL/min. The total volume of liquid flowing through the ileum over the 14-h period studied was 1168 ± 140 mL.

The highest recovery of dry matter during the study was obtained between 4 and 4.5 h after the meal (5.6 ± 0.6 g/30 min); then the dry matter flow slowly decreased, returning to the base-line level after 14 h (2.0 ± 0.3 g/30 min).

α-Glucan recovery in the ileum. The quantities of α-glucans recovered in ileal effluents during the study (expressed as % of starch ingested) are shown in Figure 3. When summing the quantities of α-glucans recovered over the 14 h, starch malabsorption was estimated at 16.5 ± 1.3% (ileal digestibility, 83.5 ± 6.6%) for the six subjects, that is, 11.3 ± 0.9 g of the 68.5 g ingested starch escaped digestion in the small intestine.

The oligosaccharides in in vivo resistant α-glucans were analyzed in pooled samples. They accounted for 9.6, 10.2 and 11.4% of the total α-glucans for subjects 1, 2 and 3, respectively. Forty (subjects 2 and 3) to fifty percent (subject 1) of the α-glucans collected in the ileal effluents were found to be still available for in vitro digestion.

Recovery of starch, non-starch polysaccharides (NSP) and proteins from the ileum. Results are given for three subjects in Table 1. The quantities of starch, non-starchy fibers and proteins collected during the study are presented in Figure 4.
of these peaks are given in Table 3 and are compared to experi-
mentally determined values for α-glucan residues after in vitro
hydrolysis. The third fraction, eluted between $K_{av} = 0.8$ and
$K_{av} = 1.0$, represented ~10% of all the α-glucans, and corre-
sponded to oligosaccharides (DP 1 to 8).

Acidic hydrolysis (l因nerization) of the cooked beans pro-
duced molecules of $DPn \approx 22$ (SEC 1), as shown by Table 3
and Figure 5. In contrast, samples obtained after in vitro enzy-
matic treatments exhibited the same main intermediate frac-
tion ($DPn \approx 40$) as the in vivo α-glucans (Table 3). The DP
of the molecules was not influenced by breaking the cells (SEC
2) or deproteinization (SEC 3) before α-amylase hydrolysis,
or by hydrolysis of the α-1,6 linkages (SEC 4).

**Recovery of starch and non-starch polysaccharides in the
stools.** Both starch and NSP were extensively fermented in
the large intestine (Table 1). The digestibility of the starch,
after passage through the large intestine, was 98.5 ± 0.9% (6
subjects), that is, 90.9 ± 6.1% of the starch reaching the
colon was fermented at this level. It was estimated that the
volunteers consumed 1.7–5.9 g/d (3.5 ± 0.6 g/d) of non-
starch fibers in addition to those provided by the beans. It
was thus possible to calculate that of the 78.7 ± 9.3% non-
starch fibers ingested coming to the large bowel, 69.0 ± 14.9%
was fermented.

**Reliability of the results obtained by the intubation
technique: pig study**

It was assumed that a recovery of PEG over 80% after the
14 h of the study was representative of an almost total collect-
on of ileal effluents. The mean recovery of PEG for all studies
was 83.3 ± 4.9%.

**Liquid and dry matter flow through the ileum.** For the
cannulation technique, the volumes obtained, after subtrac-
tion of the perfused volumes of PEG, ranged from 14.7 ± 5.6

<table>
<thead>
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<th>TABLE 1</th>
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| Recovery of total α-glucans, neutral sugars, uronic acids and proteins ingested in the ileal digesta and the stools of three human subjects 

<table>
<thead>
<tr>
<th>α-Glucans</th>
<th>Neutral sugars</th>
<th>Uronic acids</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingested quantity</td>
<td>68.5</td>
<td>21.2</td>
<td>7.6</td>
</tr>
<tr>
<td>Subject</td>
<td>Ileum</td>
<td>Feces</td>
<td>Ileum</td>
</tr>
<tr>
<td>1</td>
<td>10.33</td>
<td>0.05</td>
<td>14.00</td>
</tr>
<tr>
<td>2</td>
<td>9.10</td>
<td>0.08</td>
<td>18.75</td>
</tr>
<tr>
<td>3</td>
<td>7.99</td>
<td>4.45</td>
<td>14.20</td>
</tr>
</tbody>
</table>

1 Ileal digesta were obtained by pooling 5% of the samples collected (for each subject) during each time period.
2 Each value is the mean of two determinations except for α-glucans ingested ($n = 7$).
TABLE 2

Levels of xylose and arabinose in different parts of beans and in the human digesta\(^{1,2}\)

<table>
<thead>
<tr>
<th>Arabinose</th>
<th>Xylose</th>
<th>Xylose/Arabinose</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/kg dry matter</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Beans (whole grain)</strong></td>
<td>49.4</td>
<td>17.5</td>
</tr>
<tr>
<td><strong>Hulls</strong></td>
<td>66.2</td>
<td>67.5</td>
</tr>
<tr>
<td><strong>Contyledon</strong></td>
<td>44.9</td>
<td>10.1</td>
</tr>
<tr>
<td><strong>Ileal digesta</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Subject</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>54.5</td>
<td>13.0</td>
</tr>
<tr>
<td>2</td>
<td>64.6</td>
<td>14.4</td>
</tr>
<tr>
<td>3</td>
<td>49.3</td>
<td>12.3</td>
</tr>
</tbody>
</table>

1 Ileal digesta were obtained by pooling 5% of the samples collected for each subject during each time period.
2 Each value is the mean of two determinations.

The fraction of resistant starch was found to be 7.9\(\pm\)0.4 and 2.7\(\pm\)0.8% of the total \(\alpha\)-glucans ingested for the cannulation and intubation studies, respectively, that is, \(\sim\)5.4 g (cannulation) and 1.8 g (intubation) of the 68.5 g ingested starch were recovered at the end of the small intestine of pigs.

**Analysis of resistant \(\alpha\)-glucans obtained.** Size exclusion chromatography allowed the DP of the \(\alpha\)-glucans collected by the two methods (4 cannulation, 4 intubation studies) to be compared (Fig. 6). The profiles obtained did not really differ, except that the \(\alpha\)-glucans concentration was higher in the samples collected from cannulated pigs. The DPmax, DPn and DPw of the main fraction of \(\alpha\)-glucans (peak at K\(\text{av}\) = 0.5) were \(\sim\) 59, 47 and 69 for cannulation and \(\sim\) 55, 42 and 59 for intubation studies, respectively.

**DISCUSSION**

**Quantitative aspects.** Starch malabsorption varies widely with the source of carbohydrate, as shown by Englyst and Cummings (1987). Our results obtained in humans show that 11.3 g out of the 68.5 g of ingested starch from home-cooked white beans (Phaseolus vulgaris L.) (16.5%) is not digested in the small intestine. It has to be emphasized that this value is very close to the level of resistant starch (17.1%) predicted by the in vitro method of Englyst et al. (1986). It is slightly higher than the values for “bean flakes” reported by Schweizer et al. (1990) in ileostomates (9–11%) and by Tovar et al. (1992) for bean flour in rats (10%). The foods used in these last studies were end-products of intensive mechanical/thermic processes, which might have increased the accessibility and the digestibility of starch. The value observed in this study for starch malabsorption is closer to that obtained by Wolever et al. (1986), who found in ileostomates that 21% of the starch from whole lentils was recovered in stomal effluents.

**FIGURE 5** Size exclusion chromatography profile of an \(\alpha\)-glucan ileal residue from subject 3 and of a lintner residue of cooked beans. The ileal residue was made by pooling 5% of the samples collected during each time period. The lintner curve was obtained from one sample of lintnerized starch from beans. K\(\text{av}\), the coefficient of elution, is defined by \((V_e - V_0)/(V_t - V_0)\) where \(V_e\) is the elution volume of the sample, and \(V_0\) and \(V_t\) are the exclusion volume and the total volume of the column, respectively.
Although it could be expected that recovery of dietary fibers in the intestinal effluents would be total, only 61.0% of the uronic acids and 73.7% of the neutral sugars ingested were recovered at the end of the small intestine in humans. About one half of the uronic acids belonged to the pectins of the cotyledon cell walls (Champ et al. 1986). As was previously shown (Kon 1968), these pectins are partially solubilized during cooking of dry beans. Indeed, light microscopy gave evidence of the disappearance of middle lamella, separating the cells, after the cooking treatment. The second half of the uronic acids arose from the bean hulls (Champ et al. 1986). The selectivity of the ileal sampling using a catheter (i.d., 3 mm), which probably excludes part of the largest particles, may then explain the low recovery of neutral sugars and especially of uronic acids in the human digesta. This hypothesis was confirmed first by the comparison of the xylose/arabinose ratios in the digesta and beans. It reflects the relative excretion of hulls and cotyledons, with xylose present at a higher concentration in the outer part of the seed. The xylose/arabinose ratios fell from 0.35 in the whole cooked beans to 0.22±0.25 in the pooled samples of human digesta, which is very close to the specific ratio in the cotyledon of the cooked seeds (0.22).

Further evidence of the selectivity of the sampling using the catheter was confirmed by the study in pigs. In this study, the intubation technique was compared with ileal cannulation. This last technique allows a complete collection of the ileal digesta. Indeed, in pigs the xylose/arabinose ratios (0.20±0.27) were low, as in humans, when samples were collected using the catheter, whereas for the cannulation method, the ratio was closer to that in the whole grain (0.35). However, it is possible that this phenomenon of selectivity is minimized in humans because of more efficient chewing, which should decrease particle size and reduce sampling heterogeneity. The total absence of chewing in pigs should indeed influence the efficiency of grinding of the particles.

In the pig study, the total quantity of starch collected during the trial was also greater for the cannulation (5.38 g) than for the intubation method (1.82 g). This suggests that as well as being selective, collection is not total and leads to an underestimation of the amount of RS. This may be counterbalanced in part in the human study by the fact that nasal intubation may accelerate transit (Read et al. 1983) and decrease the time of contact between starch and digestive enzymes, leading to an overestimation of starch malabsorption.

Despite these quantitative differences between the method of intubation and cannulation in pigs, the SEC analysis of the starch collected by both techniques showed that it consisted mainly of α-glucans molecules of 45 to 60 glucose units (DPn 42 to 47). The intubation technique is therefore not ideal; however, with regard to starch, collected samples are representative of the fraction reaching the large intestine.

**Qualitative aspects.** In this study, 40–50% of the in vivo resistant starch was still potentially digestible by in vitro hydrolysis. Tovar et al. (1992) also observed such a fraction in the digesta collected from rats; it represented 30–40% of the RS from bean flour. This fraction of potentially available starch may correspond to starch fragilized, or partly solubilized during the passage through the gastrointestinal tract, whereas starch resistant to in vitro hydrolysis probably corresponds mostly to retrograded amylose. Ten percent of the RS was composed of oligosaccharides (1 to 10 glucose units). Faisant et al. (1993 and 1995) have found a similar level of oligosaccharides (13%) in the products of digestion of different starchy foods. These sugars may be present in the digesta because they transited too quickly to be absorbed. In addition, it is possible that the capacities of hydrolysis and absorption of the ileum are too poor to compensate for the fast transit time of the first part of the bolus.

The profile of non-starch fibers and proteins recovered at the end of the small intestine during the human study followed almost the same pattern as that of starch. The maximal quantity was obtained between 2.5 and 5.5 h after the beginning of the meal. In the case of proteins, a low variation in the quantities collected in the different partial pools was observed. This is certainly due to the regular recovery of proteinaceous materials of endogenous origin in the effluents collected. Cummings and MacFarlane (1991) estimated that pancreatic enzymes and gut secretions may represent 4–6 g of the 6–18 g total proteins entering the colon daily. Proteins, which are an important substrate for colonic fermentation (MacFarlane et al. 1992), were found to amount to 12±3 g in the ileal contents collected over the period of study.

Microscopic examination of the digestive effluents showed that some cotyledon cells preserved their integrity during the passage through the gut. Their proportion decreased along the experiment. We hypothesize that prolonged mechanical action of the stomach and acidity might lead to the breakdown of the cell wall, leaving starch and proteins available for digestion. In this study, the fact that a small portion of residual starch was...

**TABLE 4**

<table>
<thead>
<tr>
<th></th>
<th>Pool 2 (2.5–5.5 h)</th>
<th>Pool 3 (5.5–8.5 h)</th>
<th>Pool 4 (8.5–11 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cannulation</td>
<td>0.28 ± 0.02</td>
<td>0.30 ± 0.05</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>Intubation</td>
<td>0.27 ± 0.02</td>
<td>0.21 ± 0.01</td>
<td>0.20 ± 0.02</td>
</tr>
</tbody>
</table>

1. Ileal contents were “partial timed pools” composed of fractions collected over a 2- to 3-h period.
2. Values are means ± SEM.
3. n = 7; 7 measurements made on 3 pigs.
4. n = 5; 5 measurements made on 2 pigs.

**FIGURE 6** Size exclusion chromatography profile of α-glucans collected by the cannulation and the intubation techniques from a pig. The ileal residues were made by pooling 5% of the samples collected during each time period. Each curve is a single chromatogram (one pig). Curves are presented as examples because they are representative of all of the chromatograms. See legend to Figure 5 for definition of Kav.
present in an “encapsulated” form and that we could not observe any free starch granules outside the cells suggests that the cell wall may play a role in protecting starch from digestion through impeding the access of digestive enzymes. The cell walls have been shown in vitro to greatly decrease the accessibility of enzymes to starch (Englst et al. 1986, Thorne et al. 1983, Tovar et al. 1992, Wong and O’Dea 1983). Furthermore, Livesey et al. (1995) demonstrated the importance of the cell walls in the digestion of starch from barley in ileostomates.

In this study, the in vitro experiments suggest that entrapment of the starch in the cell walls or in the protein matrix has little effect on the products of digestion (Table 3). In vitro amylolysis produced residual molecules of DP ≈ 41, which corresponds to the major (75%) α-glucan products found in the human ileal digesta. Neither previous breakdown of the cell walls by milling under liquid nitrogen nor deproteinization changed the structural features of the residual molecules. Entrapment of starch may therefore affect the rate and extent of digestion, but does not appear to change the end-products of digestion. The residual molecules were not further degraded when adding pullulanase to α-amylase, which strongly suggests that they consisted of α-1,4 unbranched amylose chains.

The presence of the B pattern in the X-ray analysis of the cooked beans prepared “as eaten” showed the occurrence of retrograded amylose, which is known to resist digestion. Legume seeds are often rich in amylose (up to 45% of the starch) and are therefore more likely to contain higher amounts of retrograded amylose after cooking. Crystallites of pure retrograded amylose were prepared by liqtenization (Faisant et al. 1993) from the cooked beans (SEC 1) and compared with the residual α-glucan molecules of the ileal digesta. The latter had a higher DP (≈41) as opposed to the DP ≈ 20 for the crystallites of pure retrograded amylose from beans. Therefore, retrogradation alone cannot explain the undigestibility of the α-glucans recovered from the digesta. It is possible that the gelatinization of the starch on cooking might have been limited by the entrapment of the starch in the cells (Würsch et al. 1986). If this was the case, only a fraction of the starch could have retrograded. Thus, the main fraction of resistant α-glucans would correspond to the crystalline native starch resistant to digestion, with some retrograded fractions. SEC analysis of the in vivo resistant starch also showed that 15% of the RS was composed of large molecules (>400 glucose units), which may have been protected by cell walls during the digestion process.

**Fermentation in the Large Bowel.** Stool analysis showed that RS, as well as the fibers, was well fermented in the large intestine of humans. Digestibility of the starch ingested was 98.5 ± 0.9% after the passage in the large bowel. Molis et al. (1992), with pure retrograded starch, and Faisant et al. (1995), with banana starch, also found that starch was almost completely digested after passage in the large bowel (99.5 ± 0.2 and 98.7 ± 0.3%, respectively). The fermentation was certainly made easier by the partial degradation that occurs in the small intestine. The level of fermentation was similar for five subjects (97.2 ± 1.2%). One subject, however, had a relatively poor capacity of fermentation (44.2%). A large recovery of whole intact beans in the stool was observed for this subject during the stool collection. This was probably due to inefficient chewing and subsequent low degradation of the structures of the beans during the passage through the large intestine. This emphasizes the role of physical structure of the food in the digestion process. Most of the antinutrient components present in legumes are removed during classical cooking treatments (Abbas et al. 1987, Bishnoi and Khetarpaul 1993, Khokhar and Chauhan 1986) and therefore cannot be responsible for low digestion of starch.

The quantity of the starch reaching the colon was 11.3 ± 0.9 g (16.5 ± 1.3% of the 68.5 g ingested starch) where it was largely fermented (90.9 ± 6.1%). A large part of the starch from home-cooked white beans is not digested in the small intestine of healthy humans. The quantity of nondigested starch was determined to be 16.5% of the total ingested starch. Although the use of the intubation method for collecting ileal effluents in humans was found to underestimate the level of resistant starch, it was possible to analyze the residual starch collected. RS was shown to consist in part of physically inaccessible starch, but in vitro analysis demonstrated that the main fraction was composed of starch degradation products of DP 40 to 60. Retrogradation was not found to be the main factor responsible for starch malabsorption. We conclude that white beans may contain a large amount of RS formed mainly by partially degraded molecules protected by the cell walls during their transit through the gut. Finally, assuming that resistant starch has some beneficial health effects in humans, legumes may be of particular interest as foods containing high amounts of RS.

**LITERATURE CITED**


