Fate of U-^{14}C-Gelatinized and U-^{14}C-Retrograded Bean Starch in the Rat

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The fate of retrograded and gelatinized bean starch in the rat was followed using an *in vivo* radiolabel technique. [U-^{14}C]Bean starch was obtained by incubating excised unripe pods of broad beans with $^{14}CO_2$. After extraction and purification, [^{14}C]bean starch was either retrograded or gelatinized and fed to rats by gavage. Rats were housed individually in metabolic cages and were given free access to food and water. After 3 and 18 h of gavage, rats were killed by cervical dislocation and the radioactivity was measured in gut contents, tissues, feces, urine, and CO_2. The rate and extent of degradation of [^{14}C]starch in the small intestine were lower for ^{14}C-retrograded starch, as indicated by the high amounts of α-glucans of degree of polymerization >70 in the distal fraction of the small intestine 3 h after gavage. After 18 h of gavage, the percentage of radioactivity present in tissues, feces, and urine was higher in rats fed ^{14}C-retrograded starch. The [^{14}C]starch degradation products were mainly metabolized by the carcass, pelt, liver and intestinal tissues.

Keywords: *Retrograded starch; gelatinized starch; small intestine degradation; starch metabolism*

INTRODUCTION

Starch represents the major source of carbohydrates in the human diet. The interest in starch was developed when a fraction resistant to hydrolysis by salivary and pancreatic amylase (resistant starch) was shown to reach the colon and have physiological effects similar to fermentable fibers (Englyst and Cummings, 1987; Björck *et al.,* 1986). Starch was found later to be the major carbohydrate component present in the cecum of human victims of sudden death (Cumming *et al.,* 1990).

The amount of starch not digested in the jejunum has been estimated to be in the range of 3−30%, depending on the type of starch (Stephen *et al.,* 1983; Englyst and Cummings, 1987; Björck and Siljeström, 1992). Legume starches are particularly resistant to enzymatic digestion (Tovar *et al.,* 1990). Tovar *et al.* (1992) reported that 8−10% of the starch from bean and lentil precooked flours was not digested in the small intestine of rats.

Starches are known to differ in the rate of digestion in the small intestine and therefore in their metabolic responses. Slowly digested starches cause a slow glucose release and a lower glycemic and insulinemic response (Bornet *et al.,* 1989; Holm and Björck, 1992) and may influence carbohydrate and lipid metabolism by their susceptibility to bacterial fermentation (Morand *et al.,* 1992).

The rate and extent of starch digestion by amylolytic enzymes depends on intrinsic factors such as granule morphology (Colonna *et al.,* 1988, 1992), amylose/amylopectin ratio (Holm and Björck, 1988), and interaction with other food components such as cell walls (Snow and O'Dea, 1981; Würsch et *al.,* 1986), proteins (Jenkins *et al.,* 1987), and lipids (Holm *et al.,* 1983). Food processing affects availability of starch to enzyme degradation through modification of physicochemical properties. This may be the main determinant to starch digestibility since most starchy foods are processed before ingestion.

Gelatinization of starch granules during cooking in an excess of water makes starch more readily cleaved by amylolytic enzymes (Holm *et al.,* 1988). Glucose is then the principal end product of enteric starch digestion and supplies energy to the body. However, gelatinization followed by cooling results in retrogradation of starch (mainly to small aggregates of highly hydrogen-bonded amylose), making starch highly resistant to pancreatic α-amylase digestion (Ring *et al.,* 1988; Sievert and Pomeranz, 1989).

On reaching the large intestine, the resistant fraction is fermented by anaerobic bacteria (Faulks *et al.,* 1989) with the production of short-chain fatty acids, other carboxylic acids, and gases. The end products of fermentation may be metabolized or absorbed by the gut mucosa and transported to the liver and peripheral tissues by the portal blood system (Cummings and Macfarlane, 1991).

The aim of this study was to investigate the fate of retrograded and gelatinized starch in the rat using uniformly labeled [C^{14}]bean starch. This technique allows a measure of the rate of degradation of starch in the gut and the incorporation of ^{14}C into rat tissues. This method has already been shown to be suitable to investigate the degradation of radiolabeled plant cell walls in the rat (Gray *et al.,* 1993; Buchanan *et al.,* 1994).

MATERIALS AND METHODS

Preparation of ^{14}C-Labeled Bean Starch. (a) $^{14}CO_2$ Labeling of Broad Beans. Half-filled pods of broad bean (*Phaseolus vulgaris* L.) previously grown in a greenhouse, were incubated in ∼10 mL of water in a desiccator (under a flood lamp with a tank of water to minimize heating) containing a fan and 10 mCi of $NaH^{14}CO_3$ (∼50 mCi/mmol) in 5 mL of

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S0021-8561(94)00743-0 CCC: $12.00 © 1996 American Chemical Society

aqueous solution to which was added 200 mg of $(COOH)_2$ to release $^{14}CO_2$. The beans were left under a $^{14}CO_2$ atmosphere for 3 days (including 2 nights of darkness). The seeds were removed, allowed to partially dry for 1 day in a fume cupboard, and freeze-dried.

(b) Extraction of [^{14}C]Starch. Seeds (10 g) were washed and left to soak overnight at 6 °C. After washing, the softened kernels were homogenized using a Waring blender for 2 min in ~40 mL of sodium chloride solution (20 g/L). The slurry was washed through a 125 μm mesh sieve to separate fiber from starch. The filtrate was left to settle for 2 h at room temperature. The resulting supernatant was decanted and the insoluble material suspended in ethanol (absolute), filtered under vacuum, and dried (Wyatt and Horn, 1988).

Starch was purified by extracting lipids using methanol/chloroform (1:2 v/v) as solvent and protein using glacial acetic acid/80% (w/w) phenol/water (2:5:1, v/v/v) (Fry, 1988).

(c) Characterization of [^{14}C]Starch. Total starch was determined by the procedure described by Siljeström and Asp (1985). Approximately 10 mg of the purified starch was dispersed, with constant mixing, in 2 mL of 2 M KOH for 30 min at room temperature. After complete solubilization, a 200 μL aliquot was taken and adjusted to pH 4.75 by using 100 μL of acetate buffer (0.4 M, pH 4.75, containing 20 mM $CaCl_2$) and ~150 μL of 2 M HCl. Samples were incubated for 30 min at 60 °C with 60 μL of amyloglucosidase (10 mg/mL; Boehringer Mannhein No. 102857). The amount of glucose liberated was measured with a glucose peroxidase reagent (Boehringer Mannhein No. 676543). Pure soluble starch (BDH Chemicals) was used as a reference. Results are expressed as polymer weight (glucose × 0.9).

The purity of the (^{14}C)-starch, after extraction and removal of lipids and proteins, was also assessed by an enzymatic and acid treatment.

The starch was suspended in 5 mL of Na_2HPO_4/NaH_2PO_4 buffer (pH 6.0, 0.08 M) (1 mg/mL) and gelatinized at 100 °C for 1 h. The sample was treated with heat-stable α-amylase (A 3302, Sigma Chemicals), 40 μL (100 °C, 30 min), and amyloglucosidase (*Aspergillus niger*; A 9913, Sigma Chemicals) 6100 units/mL, 60 μL (60 °C, 30 min) (Prosky *et al.,* 1988).

After incubation, a 0.5 mL aliquot was chromatographed for 48 h on 3MM Whatman paper, using ethyl acetate/pyridine/water (8:2:1, v/v/v) as solvent.

Strips of the paper were placed into 3 mL of scintillate (PPO (5 g/L) and POPOP (0.5 g/L) in toluene) and assayed for ^{14}C by liquid scintillation counting (Fry, 1988).

The presence of other components such as proteins and cell walls was determined by heating 5 mg of (^{14}C)-starch with 5 mL of 5 M trifluoroacetic acid (TFA) at 120 °C for 60 min. After cooling 0.5 mL of the sample were chromatographed as above.

(d) Preparation of U-^{14}C-Gelatinized and U-^{14}C-Retrograded Bean Starch. A portion of 20 mg of purified starch was suspended in distilled water (1:4, w/v) and gelatinized by autoclaving at 121 °C for 25 min. The sample was left at ~30 °C to cool and immediately fed to rats to avoid reassociation of the polymers.

Another portion of purified bean starch (1:4 w/v in water) was retrograded by autoclaving at 121 °C for 20 min followed by incubation at 6 °C overnight. After five cycles of autoclaving/cooling, samples were freeze-dried and finely ground (Sievert and Pomeranz, 1989). A 20 mg portion was fed to rats.

(e) Fermentation *in Vitro* of U-^{14}C-Resistant Bean Starch. A closed batch-culture technique under strict anaerobic conditions was used for the *in vitro* fermentation. The medium (pH 7.0) contained Tryptone (2.5 g/L) and minerals (Na_2HPO_4 1.4 g/L, KH_2PO_4 1.6 g/L, $MgSO_4 \cdot 7H_2O$ 0.2 g/L, $(NH_4)HCO_3$ 1 g/L, $NaHCO_3$ 8.7 g/L, $CaCl_2 \cdot 2H_2O$ 16.5 mg/L, $MnCl_2 \cdot 4H_2O$ 12.5 mg/L, $CoCl_2 \cdot 6H_2O$ 1.25 mg/L, and $FeCl_3 \cdot 6H_2O$ 1 mg/L) (Goering and Van Soest, 1979).

Cecal contents freshly removed from male Wistar Hans rats fed a CRM(x) diet (Chow Lab-sure, Croydon, England) (Table 1) were used as inoculum. Approximately 4 g of cecal contents was placed into 50 mL of sterile anaerobic medium (8%) and incubated with continuous agitation at 37 °C for 45 min. The slurry was filtered through a 51 μm mesh sieve and the filtrate was used for inoculation.

Table 1. Composition of Basal Diet (g/100 g of Dry Matter)[a]

protein	18.3	soluble non-starch polysaccharides[b]	3.1
fat	2.9	insoluble non-starch polysaccharides[b]	10.2
calcium	0.8	trace elements[c]	1.6
phosphorus	0.6	vitamins[d]	2.5
salt	0.7	amino acids[e]	9.1
starch	46.5		

[a] Data supplied by Special Diet Services Ltd. [b] Information supplied by H. N. Englyst, personal communication. [c] Contents (ppm): cobalt 0.4, copper 0.7, iodine 1.3, iron 30, magnesium 102, and manganese 25. [d] Contents vitamin A 8000 IU, vitamin B_1 4 mg, vitamin B_2 8 mg, vitamin B_6 6 mg, vitamin B_{12} 12 μg, vitamin D_3 1,000 IU, vitamin E 60 IU, vitamin K 10 mg, choline chloride 200 mg, folic acid 10 mg, and pantothenic acid 12 mg. [e] Contents (g/100 g of diet): arginine 1.2, cysteine 0.2, glycine 0.9, histidine 0.4, isoleucine 0.7, leucine 1.4, lysine 1.0, methionine 0.3, phenylalanine 0.8, threonine 0.6, tryptophan 0.2, tyrosine 0.6, and valine 0.8.

Four samples of 50 mg of U-^{14}C-resistant bean starch were dispensed into 15 mL Hungate tubes containing 8 mL of sterile medium plus 1 mL of reducing solution (cysteine hydrochloride 6.2 g/L, NaOH 1.6 g/L, and $Na_2S \cdot 9H_2O$ 6.2 g/L). After being gassed with CO_2, the samples were inoculated with 3 mL of filtrate and placed in an incubator at 37 °C for 24 h.

Aliquots were removed after 24 h and short-chain fatty acids analyzed by GLC (Spiller *et al.,* 1980). Controls were prepared as above without the addition of starch.

Animal Experiments. (a) Gavage of [^{14}C]Starch to Rats. Animal experiments were performed according to the Home Office Guidelines, U.K. Male Wistar Hans rats (~150 g) previously fed on a stock pelleted diet CRM(x) were used for the study (Table 1).

Rats (eight per group) were dosed by gavage with ~20 mg of either gelatinized (47.0 × 10^3 Bq) or retrograded [U-^{14}C]-bean starch (43.3 × 10^3 Bq) dispersed in 0.5 mL of distilled water. After feeding, rats were individually caged in metabolic cages, with a broad-spaced gridded floor to minimize coprophagy, and were given free access to food and water. The cage was enclosed in a Perspex chamber fitted with an outlet through which the CO_2 produced during the experiment was collected by drawing air through the cage and through ~50 mL of Carbo-Sorb (Canberra Packard, Berkshire, U.K.). Rats were housed in a room at 21 °C with a 12 h light−12 h dark cycle.

After 3 and 18 h of feeding, CO_2, urine, and feces were collected separately. Rats were killed by cervical dislocation and immediately dissected.

Stomach, small intestine (divided into three equal parts by length), cecum, and colon contents were removed; the tissues were washed in distilled water and kept at −20 °C for further analysis. The gut contents were frozen at −20 °C in 0.1 M NaOH to avoid further fermentation.

(b) Analysis of ^{14}C. A 1 mL sample of gut contents or 100 mg of feces was added to 4 mL of Optisolv (Pharmacia Wallac) and solubilized at 50 °C for 4 h. Aliquots (1 mL) of the solutions were mixed with 10 mL of scintillation fluid (Hionic-Fluor, Canberra Packard) for ^{14}C analysis.

All tissues except the carcass were weighed and samples of ~100 mg solubilized in 1 mL of Optisolv at 50 °C overnight. The carcass was previously homogenized in a Waring blender and then an aliquot of 100 mg solubilized. After complete digestion, 10 mL of Hionic-Fluor was added and samples were assayed for radioactivity.

^{14}C in urine and CO_2 was measured by adding 10 mL of scintillation fluid (Pico-Aqua, Canberra Packard) to 1 mL of sample.

Samples were measured for 20 min in a Tri-carb 4430 liquid scintillation counter. The counts were corrected for quenching (external standard) and calculated as the average of four replicates.

(c) Characterization of Starch Degradation Products. Starch degradation products in the last segment of the small

intestine were characterized by gel permeation chromatography using a Sephadex G50 SuperFine column (40 × 1 cm; Pharmacia Biotech Ltd., Milton Keynes, U.K.) eluted with 0.1 M potassium hydroxide, 20 mL/h.

The column was calibrated using linear α-glucans obtained by treatment of waxy sorghum starch with isoamylase (Hayashibara Biochemical Laboratories, Inc., Tokyo, Japan) (Glennie *et al.,* 1987).

The starch was made water soluble by mixing 10 mg of starch with 1 mL of 2 M KOH. The sample was placed in a boiling water bath for 90 min. After cooling, the starch was precipitated with 9 mL of 96% ethanol and left at 4 °C for 15 min. The sample was centrifuged at 1400*g* for 10 min. The pellet was redissolved in 1 mL of 2 M KOH and boiled for 30 min; the starch was precipitated as noted above. The sample was dissolved in 500 μL of water, an aliquot of ~5 mg of starch was added to 500 μL of acetate buffer (0.01 M, pH 3.8) and 10 μL of isoamylase (~600 units). The solution was incubated at 30 °C for 24 h. After incubation the sample was boiled for 3 min and centrifuged (1400*g*, 5 min). After cooling, 250 μL was loaded to the column.

Total carbohydrates were determined by the phenol/sulfuric acid method (Dubois *et al.,* 1956) and reducing sugars by the method of Nelson and Somogyi as described by Robyt and Whelan (1968). The degree of polymerization (DP) was calculated as the total carbohydrates divided by the reducing sugars in each of the collected fractions.

The soluble and insoluble fractions of the small intestine contents were separated by centrifugation for 10 min at 1400 g. The pellet was washed twice with distilled water and the supernatant pooled and freeze-dried. This fraction was resolubilized in 0.1 M KOH. After filtration through a Millipore filter (0.5 μm pore size), 250 μL of sample was loaded to the column.

The pellet was solubilized in 2 M KOH for 24 h with constant mixing. After dilution to 0.1 M KOH, samples were filtered through a Millipore filter (0.5 μm pore size) and an aliquot of 250 μL loaded to the column. In both samples, aliquots of 1 mL were collected and measured for radioactivity in 10 mL of Hionic-Fluor.

Total faecal α-glucans (^{14}C-undigested starch degradation products) in rats fed resistant starch were determined by mixing ~500 mg of feces in 5 mL of 2 M KOH for 24 h. After solubilization, samples were filtered and precipitated in 80% ethanol. The tubes were left at 4 °C overnight and the precipitate was isolated by centrifugation (1400*g*, 20 min). After purification by two further precipitations and centrifugations, samples were resolubilized in 1 mL of distilled water and analyzed for radioactivity in 10 mL of Pico-Aqua.

Statistical Analysis. Results are given as means ± SEM. Means were tested for significance using a 2×2 ANOVA test. Nonparametric data were assayed for significance using the Mann-Whitney test. Differences of $p < 0.05$ were considered significant. Statistical evaluation was performed with the package Minitab, release 7.1, 1989, State College, PA.

RESULTS AND DISCUSSION

Analysis of [^{14}C]Bean Starch. The total activity of the [U-^{14}C]starch granules measured after extraction and purification was 2.35 × 10^3 Bq/mg of dry material. After retrogradation the total activity of [^{14}C]starch was 2.17 × 10^3 Bq/mg of dry materials. The differences in radioactivity between raw and retrograded starches were due to differences in humidity.

The total concentration of starch in the purified sample was 95%. Enzymatic hydrolysis of the purified samples released glucose as the principal ^{14}C-labeled product (94% of total starting material). A small peak, corresponding to 3% of the total radioactivity, was detected at the origin (Figure 1A). The disappearance of this peak after TFA hydrolysis suggested that it could be cell wall material of small particle size or endogenously present proteins (Figure 1B).

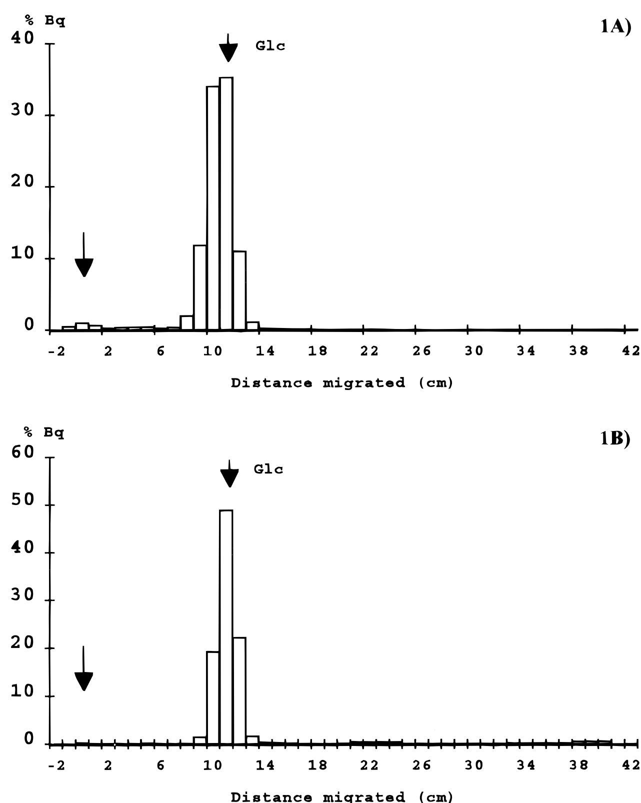

Figure 1. Paper chromatography in ethyl acetate/pyridine/water of purified [^{14}C]bean starch, after (A) α-amylase and amyloglucosidase treatment or (B) TFA treatment. The position of the marker is indicated: Glc, glucose.

Degradation of [^{14}C]Starch and Metabolism of [^{14}C]Starch Degradation Products. An important aspect of dietary starch digestion is its processing before ingestion. In this study we have compared the degradation of retrograded and gelatinized starch in the gastrointestinal tract and subsequent metabolism of their degradation products by the rat tissues using a radioactively labeled [U-^{14}C]bean starch preparation.

The low concentration of [U-^{14}C]starch required in the experiments is likely to produce minimal interference with the host diet transit time and its metabolism. The stock diet had a high concentration of non-starch and starch polysaccharides (Table 1); therefore, a high induction of cecal saccharolytic enzymes can be expected. An 18 h postgavage time period was chosen as the final time on the basis of previous work in which most [^{14}C]plant cell wall had passed through the cecum and colon and metabolized in tissues (Gray *et al.,* 1993, Buchanan *et al.,* 1994).

The total distribution of ^{14}C in gut contents, tissues, feces, urine, and CO_2 at 3 (early-absorptive period) and 18 h (postabsorptive period) after gavage of either gelatinized or retrograded [U-^{14}C]bean starch is summarized in Table 2.

Gelatinized starch was more rapidly degraded than retrograded starch, as shown by the lower amounts of radioactivity in the gut contents after 3 h of gavage. At this time, the ^{14}C incorporated into total tissues was similar in rats fed labeled gelatinized and retrograded starch.

The ^{14}C excreted in urine, feces, and gases 3 h after gavage (calculated as percentage not recovered in tissues and gut contents), was 57% in rats fed gelatinized starch and 14% in rats fed resistant starch. The percentage in urine and feces was less than 1% in both

Table 2. Distribution of ^{14}C (% Bq Gavaged) in Tissues, Gut Contents, Feces, Urine, and CO_2 3 and 18 h after Gavage of U-^{14}C-Gelatinized or U-^{14}C-Retrograded Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		retrograded	
	3 h	18 h	3 h	18 h
gut content	17.8 ± 0.7$^{a,\alpha}$	1.3 ± 0.1$^{b,\gamma}$	59.2 ± 5.2$^{c,\beta}$	2.0 ± 0.5$^{d,\gamma}$
tissues	24.9 ± 1.6 $^{a,\alpha}$	16.0 ± 0.9$^{b,\gamma}$	26.5 ± 2.3$^{c,\alpha}$	26.8 ± 1.5$^{c,\delta}$
feces	0.2 ± 0.2$^{a,\alpha}$	2.9 ± 0.1$^{b,\gamma}$	0.2 ± 0.07$^{c,\alpha}$	18.8 ± 4.6$^{d,\delta}$
urine	0.4 ± 0.01$^{a,\alpha}$	1.6 ± 0.2$^{b,\gamma}$	0.4 ± 0.2$^{c,\alpha}$	2.4 ± 0.2$^{d,\delta}$
CO_2	19.0 ± 0.6$^{a,\alpha}$	26.0 ± 1.4$^{b,\gamma}$	5.6 ± 0.8$^{c,\alpha}$	15.9 ± 0.8$^{d,\delta}$
total	62.3	47.8	91.9	65.9

[a] Values are means ± SEM for four rats. Different superscript at 3 versus 18 h for gelatinized starch (a, b) or retrograded starch (c, d), and between starches at 3 (α, β) and 18 (γ, δ) h, denotes significant differences ($p < 0.05$).

Table 3. Distribution of ^{14}C (% Bq Gavaged) in Gut Contents 3 and 18 h after Gavage U-^{14}C-Gelatinized or U-^{14}C-Resistant Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		retrograded	
	3 h	18 h	3 h	18 h
stomach	12.37 ± 0.19$^{\alpha}$	0.12 ± 0.05$^{\gamma}$	30.17 ± 2.59$^{\beta}$	0.03 ± 0.01$^{\gamma}$
SI[b] (proximal)	1.31 ± 0.02$^{\alpha}$	0.24 ± 0.03$^{\gamma}$	3.63 ± 1.03$^{\alpha}$	0.39 ± 0.08$^{\gamma}$
SI (medial)	1.47 ± 0.11$^{\alpha}$	0.21 ± 0.01$^{\gamma}$	5.96 ± 2.22$^{\alpha}$	0.41 ± 0.09$^{\gamma}$
SI (distal)	1.49 ± 0.27$^{\alpha}$	0.29 ± 0.01$^{\gamma}$	15.05 ± 1.08$^{\beta}$	0.29 ± 0.06$^{\gamma}$
cecum	0.79 ± 0.08$^{\alpha}$	0.18 ± 0.03$^{\gamma}$	3.69 ± 0.41$^{\beta}$	0.47 ± 0.23$^{\gamma}$
colon	0.36 ± 0.11$^{\alpha}$	0.23 ± 0.03$^{\gamma}$	0.69 ± 0.38$^{\alpha}$	0.37 ± 0.17$^{\gamma}$

[a] Values are means ± SEM for four rats. Different superscript for gelatinized versus retrograded starch at 3 (α, β) and 18 (γ, δ) h denotes significant differences ($p < 0.05$). [b] SI, small intestine.

cases; therefore, ~56% of the radioactivity in rats fed ^{14}C-gelatinized starch and 13% in rats fed ^{14}C-resistant starch may had been expelled as gases (carbon dioxide, methane). Although methane was not measured, the experimental values of CO_2 recorded in Table 2 suggest a low experimental recovery of the gas in both groups. The low $^{14}CO_2$ recovered in expired breath explains the low total recoveries shown in Table 2.

After 18 h, the radioactivity remaining in the gut was similar in both groups. Tissues from rats fed gelatinized starch showed a reduction in radioactivity, meanwhile the amount of ^{14}C in tissues of rats fed retrograded starch remained unchanged.

The ^{14}C excreted in urine and feces was higher in rats fed labeled resistant starch.

The amount of undigested [^{14}C]starch degradation products in feces from rats fed ^{14}C-resistant starch (as α-glucans insoluble in 80% ethanol) was ~0.1%. The presence of low amounts of resistant starch in feces indicates an extensive degradation of the retrograded starch along the large intestine. This result, along with the high amounts of radioactivity found in the feces, may suggest a possible incorporation of ^{14}C into bacterial biomass. The high effectiveness of amylomaize starch in promoting bacterial proliferation has been suggested by other authors (Rémésy and Demigné, 1989). As expected, only small amounts of products of gelatinized starch metabolism were excreted in the urine and feces, being lost by complete oxidation as CO_2.

Buchanan *et al.* (1994) have recently reported equal amounts (2.3%) of ^{14}C excreted as urine, but lower amounts (9%) of ^{14}C voided as feces using plant cell wall.

The profile of ^{14}C in gut contents, 3 and 18 h after gavage gelatinized or resistant starch, is summarized in Table 3. Results showed a slower rate and extent of digestion of starch in rats fed retrograded starch than that of rats fed gelatinized starch. After 3 h of feeding, both groups still retained most of the radioactivity in the stomach, however, the gastric emptying of the

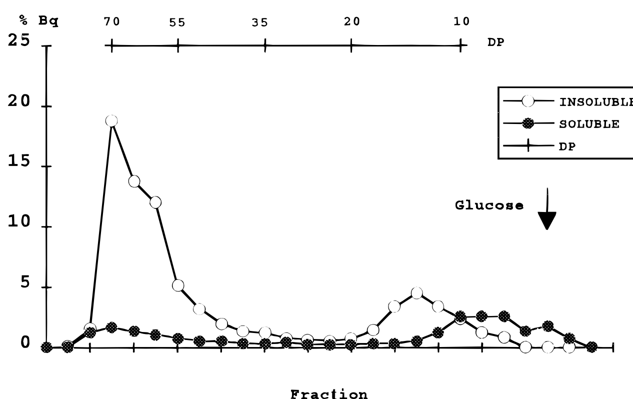

Figure 2. Gel permeation chromatography of ^{14}C-resistant bean starch degradation products (insoluble and soluble) present in the lower segment of the small intestine after 3 h of gavage.

gelatinized starch was faster than the retrograded starch. The percentage of ^{14}C left in the small intestine was uniformly distributed along the intestine in rats fed gelatinized starch. The pattern differed along the small intestine in rats fed resistant starch, increasing from the upper, to the lower segment. The amounts of ^{14}C in the cecum was also higher in the latter group. The distribution of ^{14}C left in the gastrointestinal tract after 18 h of gavage did not differ between the two groups studied.

The slower rate and extent of digestion of starch in rats fed labeled retrograded starch was accounted for partly by the more gradual gastric emptying of resistant starch, which extended the time course of starch available for pancreatic α-amylase digestion, and partly by the resistance of labeled retrograded starch to amylase degradation in the small intestine, as indicated by the presence of large amounts of ^{14}C in the lower segment of the small intestine and the cecum.

The slow rate of degradation in the small intestine seems to be related to the intrinsic properties of the

Table 4. Distribution of ^{14}C (% Bq Gavaged) in Gut Tissues 3 and 18 h after Gavage of U-^{14}C-Gelatinized or U-^{14}C-Resistant Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		retrograded	
	3 h	18 h	3 h	18 h
stomach	0.36 ± 0.04^{a,a}	0.10 ± 0.03^{b,g}	0.30 ± 0.04^{c,a}	0.14 ± 0.02^{d,g}
SI[b] (proximal)	0.53 ± 0.01^{a,a}	0.31 ± 0.02^{b,g}	0.46 ± 0.01^{c,a}	0.46 ± 0.03^{c,d}
SI (medial)	0.38 ± 0.05^{a,a}	0.25 ± 0.01^{a,g}	0.41 ± 0.06^{c,a}	0.42 ± 0.07^{c,d}
SI (distal)	0.39 ± 0.05^{a,a}	0.29 ± 0.02^{a,g}	0.45 ± 0.05^{c,a}	0.40 ± 0.02^{c,d}
cecum	0.11 ± 0.01^{a,a}	0.08 ± 0.01^{a,g}	0.18 ± 0.01^{c,b}	0.13 ± 0.01^{d,d}
colon	0.30 ± 0.01^{a,a}	0.14 ± 0.02^{a,g}	0.30 ± 0.04^{c,a}	0.27 ± 0.02^{c,d}
total	2.07	1.17	2.10	1.82

[a] Values are means ± SEM for four rats. Different superscript at 3 versus 18 h for gelatinized starch (a, b) or retrograded starch (c, d), and between starches at 3 (α, β) and 18 (γ, δ) h, denotes significant differences ($p < 0.05$). [b] SI, small intestine.

Table 5. Distribution of ^{14}C [Bq (g of Wet Tissue)$^{-1}$] in Gut Tissues 3 and 18 h after Gavage of U-^{14}C-Gelatinized or U-^{14}C-Resistant Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		retrograded	
	3 h	18 h	3 h	18 h
stomach	107 ± 8$^{a,\alpha}$	39 ± 3$^{b,\gamma}$	102 ± 6$^{c,\alpha}$	52 ± 9$^{d,\gamma}$
SI[b] (proximal)	144 ± 11$^{a,\alpha}$	56 ± 1$^{b,\gamma}$	109 ± 25$^{c,\alpha}$	86 ± 16$^{c,\gamma}$
SI (medial)	104 ± 16$^{a,\alpha}$	55 ± 1$^{a,\gamma}$	109 ± 5$^{c,\alpha}$	85 ± 15$^{c,\gamma}$
SI (distal)	109 ± 5$^{a,\alpha}$	60 ± 11$^{a,\gamma}$	116 ± 9$^{c,\alpha}$	85 ± 15$^{c,\gamma}$
cecum	58 ± 1$^{a,\alpha}$	36 ± 3$^{b,\gamma}$	83 ± 8$^{c,\beta}$	52 ± 5$^{d,\gamma}$
colon	55 ± 2$^{a,\alpha}$	35 ± 3$^{a,\gamma}$	83 ± 15$^{c,\alpha}$	73 ± 9$^{c,\gamma}$

[a] Values are means ± SEM for four rats. Different superscript at 3 versus 18 h for gelatinized starch (a, b) or retrograded starch (c, d), and between starches at 3 (α,β) and 18 (γ,δ) hours denote significant differences ($p < 0.05$). Results assayed for significance using Mann-Whitney test. [b] SI, small intestine.

Table 6. Distribution of ^{14}C (% Bq Gavaged) in Carcass, Pelt, and Liver 3 and 18 h after Gavage of U-^{14}C-Gelatinized or U-^{14}C-Resistant Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		retrograded	
	3 h	18 h	3 h	18 h
carcass	12.87 ± 0.64$^{a,\alpha}$	7.06 ± 0.57$^{b,\gamma}$	12.66 ± 0.55$^{c,\alpha}$	11.00 ± 1.00$^{c,\delta}$
liver	2.52 ± 0.09$^{a,\alpha}$	1.18 ± 0.04$^{b,\gamma}$	3.19 ± 0.54$^{c,\alpha}$	3.08 ± 0.42$^{c,\delta}$
pelt	5.20 ± 0.14$^{a,\alpha}$	5.20 ± 0.08$^{a,\gamma}$	6.48 ± 1.00$^{c,\alpha}$	9.04 ± 0.62$^{c,\delta}$
total	20.59	13.44	22.33	23.12

[a] Values are means ± SEM for four rats. Different superscript at 3 versus 18 h for gelatinized starch (a, b) or retrograded starch (c, d), and between starches at 3 (α, β) and 18 (γ, δ) h, denotes significant differences ($p < 0.05$).

retrograded starch (degree of retrogradation) since other food components were extracted from the beans before treatment.

The characterization of degradation products of ^{14}C-labeled retrograded starch in the distal fraction of the small intestine by gel permeation chromatography (Figure 2) showed that, after 3 h of feeding, the predominant radioactive fraction was made of insoluble α-glucans with a maximum peak of DP ≥ 70 (60%) and a smaller fraction of DP ≤ 20 (11%). A soluble fraction of oligosaccharides was also found (11%).

The resistance of retrograded starches to α-amylolysis is already well established (Ring *et al.,* 1988; Colonna *et al.,* 1992). This factor controls the rate of appearance of glucose in the small intestine and has been found to be a determining factor in metabolic effects of starches, such as glycemia and insulinemia in humans (Bornet *et al.,* 1989).

Faisant *et al.* (1993a,b) have shown that the starch remnants present in ileal contents was constituted mainly of retrograded amylose of an average DP of 35 along with smaller amounts of high molecular weight semicrystalline α-glucans and soluble oligosaccharides of DP 5-1. The presence of glucose in the lower parts of the small intestine may have an important metabolic response since its absorption at different sites in the small intestine has been suggested to elicit differing hormonal responses (Livesey *et al.,* 1994) and could be responsible for the trophic effect on the distal small intestine found in rats fed retrograded amylose (Faulks *et al.,* 1989; Gee *et al.,* 1991).

Gelatinized starch is taken up as glucose in the small intestine, while retrograded starch can be taken up as glucose in the small intestine and as short-chain fatty acids (SCFAs) in the large intestine. Glucose and SCFAs are expected to be metabolized differently by the rat.

The incorporation of ^{14}C in gut tissues after degradation of the starches is shown in Table 4 (as % Bq gavaged) and Table 5 [as Bq (g of wet wt tissue)$^{-1}$]. After 3 h of gavage, both groups had incorporated similar amounts of radioactivity (~2%). It is noteworthy the high levels of radioactivity incorporated in the upper small intestine in rats fed gelatinized starch, where most absorption occurs for rapidly digestible starches (Faulks *et al.,* 1992). The distribution of radioactivity in the three fractions of the small intestine was more homogeneous and significantly higher after 18 h in rats fed retrograded starch.

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Table 7. Distribution of ^{14}C [Bq (g of Wet Tissue)$^{-1}$] in Carcass, Pelt, and Liver 3 and 18 h after Gavage of U-^{14}C-Gelatinized or U-^{14}C-Resistant Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		retrograded	
	3 h	18 h	3 h	18 h
carcass	$54 \pm 3^{a,\alpha}$	$29 \pm 3^{b,\gamma}$	$73 \pm 13^{c,\alpha}$	$50 \pm 4^{c,\delta}$
liver	$101 \pm 2^{a,\alpha}$	$45 \pm 1^{b,\gamma}$	$128 \pm 13^{c,\alpha}$	$129 \pm 14^{c,\delta}$
pelt	$52 \pm 2^{a,\alpha}$	$51 \pm 1^{a,\gamma}$	$71 \pm 9^{c,\alpha}$	$90 \pm 3^{c,\delta}$

[a] Values are means ± SEM for four rats. Different superscript at 3 versus 18 h for gelatinized starch (a, b) or retrograded starch (c, d), and between starches at 3 (α, β) and 18 (γ, δ) h, denotes significant differences ($p < 0.05$). Results assayed for significance using Mann-Whitney test.

Table 8. Distribution of ^{14}C (% Bq Gavaged) in Tissues 3 and 18 h after Gavage of U-^{14}C-Gelatinized or U-^{14}C-Resistant Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		retrograded	
	3 h	18 h	3 h	18 h
adrenal	$0.02 \pm 0.01^{a,\alpha}$	$0.03 \pm 0.01^{a,\gamma}$	$0.04 \pm 0.01^{c,\beta}$	$0.03 \pm 0.01^{c,\gamma}$
brain	$0.40 \pm 0.01^{a,\alpha}$	$0.17 \pm 0.01^{b,\gamma}$	$0.43 \pm 0.03^{c,\alpha}$	$0.24 \pm 0.06^{c,\gamma}$
bladder	$0.04 \pm 0.01^{a,\alpha}$	$0.01 \pm 0.01^{b,\gamma}$	$0.04 \pm 0.01^{c,\alpha}$	$0.01 \pm 0.01^{c,\gamma}$
fat	$0.39 \pm 0.07^{a,\alpha}$	$0.41 \pm 0.02^{a,\gamma}$	$0.31 \pm 0.06^{c,\alpha}$	$0.38 \pm 0.15^{c,\gamma}$
heart	$0.19 \pm 0.03^{a,\alpha}$	$0.08 \pm 0.01^{b,\gamma}$	$0.17 \pm 0.01^{c,\alpha}$	$0.08 \pm 0.04^{c,\gamma}$
kidney	$0.20 \pm 0.03^{a,\alpha}$	$0.14 \pm 0.01^{a,\gamma}$	$0.31 \pm 0.03^{c,\alpha}$	$0.23 \pm 0.03^{c,\gamma}$
lung	$0.23 \pm 0.01^{a,\alpha}$	$0.11 \pm 0.01^{b,\gamma}$	$0.20 \pm 0.01^{c,\alpha}$	$0.19 \pm 0.02^{c,\gamma}$
pancreas	$0.22 \pm 0.05^{a,\alpha}$	$0.08 \pm 0.01^{a,\gamma}$	$0.21 \pm 0.02^{c,\alpha}$	$0.08 \pm 0.01^{d,\gamma}$
spleen	$0.12 \pm 0.01^{a,\alpha}$	$0.10 \pm 0.01^{a,\gamma}$	$0.13 \pm 0.01^{c,\alpha}$	$0.16 \pm 0.03^{c,\gamma}$
thymus	$0.13 \pm 0.01^{a,\alpha}$	$0.11 \pm 0.01^{a,\gamma}$	$0.14 \pm 0.01^{c,\alpha}$	$0.15 \pm 0.02^{c,\gamma}$
testes	$0.35 \pm 0.03^{a,\alpha}$	$0.17 \pm 0.01^{b,\gamma}$	$0.57 \pm 0.13^{c,\alpha}$	$0.34 \pm 0.06^{c,\gamma}$
total	2.29	1.41	2.55	1.89

[a] Values are means ± SEM for four rats. Different superscript at 3 versus 18 h for gelatinized starch (a, b) or retrograded starch (c, d), and between starches at 3 (α, β) and 18 (γ, δ) h, denotes significant differences ($p < 0.05$).

Table 9. Distribution of ^{14}C [Bq (g of Wet Tissue)$^{-1}$] in Tissues 3 and 18 h after Gavage of U-^{14}C-Gelatinized or U-^{14}C-Resistant Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		retrograded	
	3 h	18 h	3 h	18 h
adrenal	$115 \pm 20^{a,\alpha}$	$124 \pm 16^{a,\gamma}$	$226 \pm 9^{c,\beta}$	$167 \pm 17^{d,\gamma}$
brain	$82 \pm 2^{a,\alpha}$	$32 \pm 1^{b,\gamma}$	$86 \pm 7^{c,\alpha}$	$60 \pm 6^{c,\delta}$
bladder	$72 \pm 8^{a,\alpha}$	$31 \pm 2^{b,\gamma}$	$86 \pm 3^{c,\alpha}$	$54 \pm 19^{c,\gamma}$
fat	$117 \pm 10^{a,\alpha}$	$136 \pm 24^{a,\gamma}$	$143 \pm 12^{c,\alpha}$	$127 \pm 42^{c,\gamma}$
heart	$90 \pm 14^{a,\alpha}$	$35 \pm 5^{a,\gamma}$	$78 \pm 5^{c,\alpha}$	$48 \pm 9^{c,\gamma}$
kidney	$66 \pm 3^{a,\alpha}$	$32 \pm 1^{b,\gamma}$	$81 \pm 9^{c,\alpha}$	$57 \pm 8^{c,\gamma}$
lung	$55 \pm 3^{a,\alpha}$	$27 \pm 3^{b,\gamma}$	$59 \pm 2^{c,\alpha}$	$54 \pm 11^{c,\gamma}$
pancreas	$119 \pm 9^{a,\alpha}$	$41 \pm 1^{b,\gamma}$	$124 \pm 8^{c,\alpha}$	$44 \pm 3^{d,\gamma}$
spleen	$75 \pm 5^{a,\alpha}$	$49 \pm 1^{a,\gamma}$	$75 \pm 3^{c,\alpha}$	$92 \pm 22^{c,\gamma}$
thymus	$79 \pm 1^{a,\alpha}$	$59 \pm 2^{a,\gamma}$	$84 \pm 1^{c,\alpha}$	$91 \pm 16^{c,\gamma}$
testes	$54 \pm 8^{a,\alpha}$	$23 \pm 1^{a,\gamma}$	$73 \pm 17^{c,\alpha}$	$51 \pm 7^{c,\gamma}$

[a] Values are means ± SEM for four rats. Different superscript at 3 versus 18 h for gelatinized starch (a, b) or retrograded starch (c, d), and between starches at 3 (α, β) and 18 (γ, δ) h, denotes significant differences ($p < 0.05$). Results assayed for significance using the Mann−Whitney test.

The ^{14}C in the cecal tissue after 3 h of gavage, and in the cecal and colon tissues after 18 h, was higher in rats fed the retrograded starch.

The *in vitro* fermentation of ^{14}C-retrograded starch produced SCFAs in a molar ratio (acetic/propionic/butyric) (3.9:1:1.7; mmol/L 59:15:26). The high production of butyrate could explain the rapid incorporation of radioactivity into cecal tissue. The selective absorption and metabolism of butyrate by the epithelium in the large bowel has been reported (Roediger, 1982).

The incorporation of ^{14}C into individual tissues after 3 and 18 h of gavage gelatinized or resistant bean starch is outlined in Tables 6−9.

The carcass, pelt, and liver showed the highest uptake in both cases (Tables 6 and 7). The remaining tissues contributed only 2.3% in rats fed gelatinized starch and 2.5% in rats fed resistant starch 3 h after gavage (Table 8). After 18 h, rats fed gelatinized starch had a decrease in radioactivity in almost all tissues. Rats fed retrograded starch showed a depletion of ^{14}C only in the pancreas (Tables 6 and 8).

The utilization of volatile fatty acids by tissues is dependent on activation by fatty acyl-CoA synthetases, which are present in many tissues including the liver, muscle, brain, gut mucosa, and adipose tissue (Groot *et al.,* 1974). The substitution of gelatinized (glucose) for retrograded starch (SCFAs) significantly increased the labeling in the adrenal glands during the early-absorptive period, and in the carcass, liver, and pelt, 18 h postgavage (Tables 6 and 8). A high incorporation of radioactivity into liver and pelt tissues has been also shown by Buchanan *et al.* (1994) using uniformly ^{14}C-labeled spinach plant cell walls as fermentation substrate.

No significant differences were found in body fat (Tables 8 and 9), possibly due to the relatively low activity of acetyl-CoA synthetase in rat adipose tissue (Knowles *et al.,* 1974). De Deckere *et al.* (1993) have observed a reduction on fat accretion in rats fed retrograded amylose-rich cornstarch.

In summary, this study shows that the resistance of ^{14}C-resistant bean starch to amylolytic degradation in the small intestine compared to ^{14}C-gelatinized starch provides the body with a supply of starch degradation products available for microbial fermentation contributing to variations in the metabolism of the liver, carcass, pelt, and gut tissues in the rat. A contribution to bacterial biomass may be an important feature of retrograded bean starch fermentation.

ACKNOWLEDGMENT

We thank Janice Miller (Centre for Plant Science) for excellent technical assistance and the staff of the Biomedical Research Facility (Western General Hospital) for their help with the animal experiments.

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Received for review December 30, 1994. Accepted May 22, 1996.[⊗] The financial support of the Spanish comisión Interministerial de Ciencia y Tecnología (Project ALI 92-0278) and the Spanish Ministry of Education and Science are acknowledged. This investigation was performed within the EURESTA program.

JF9407432

[⊗] Abstract published in *Advance ACS Abstracts,* July 1, 1996.