Metabolic Transformations of U-^{14}C-Resistant and U-^{14}C-Gelatinized Bean Starch in Rat Liver and Body

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The metabolic fate of carbon derived from dietary U-^{14}C-resistant (RS) and U-^{14}C-gelatinized (GS) bean starches was studied in rat liver and body (mainly muscle). Male Wistar Hans rats were fed 20 mg of either GS (43.3×10^3 Bq) or RS (47.3×10^3 Bq) by gavage. The incorporation of ^{14}C was measured in the amino acid, lipid, and glycogen fractions of the liver and carcass. The substitution of U-^{14}C-gelatinized starch with U-^{14}C-resistant starch provided the body with ^{14}C incorporated mainly into proteins and to a lesser extent into lipids in both liver and carcass. Glutamic and aspartic acids and alanine were the amino acids with highest incorporation of ^{14}C in the liver. Feeding of U-^{14}C-resistant starch led to a high radioactivity in the phospholipid, cholesterol, and triacylglycerol fractions in the liver and to a lesser extent in cholesterol and the free fatty acids fraction in carcass.

Keywords: *Resistant starch; rat metabolism; short-chain fatty acids; protein; lipid*

INTRODUCTION

It is well established that resistant starch in not hydrolyzed by salivary and pancreatic amylases in humans (Englyst and Cummings, 1987) and rats (Björck *et al*., 1986). This fraction passes into the cecum and is fermented by the bacterial microflora.

The amount and structural and physicochemical properties of the starch fractions reaching the caecum are very varied and depend on the botanical source and hydrothermic processing before feeding. Gelatinization during cooking and food processing destroys the starch granules, making the starch polymers readily available to pancreatic α-amylases (Holm *et al*., 1988). In contrast, autoclaving followed by cooling results in the recrystallization of amylose and amylopectin (Miles *et al*., 1985). In this form, starches (retrograded starch) are resistant to digestion in the small intestine (Faulks *et al*., 1989; Björck and Siljeström, 1992).

Starch fermentation favors the production of butyric (Englyst and Macfarlane, 1986, Englyst *et al*., 1987, Weaver *et al*., 1992) and lactic acids (Andrieux *et al*., 1992) compared to non-starch polysaccharides. However, several studies have shown that variations in the source of starch and microbial population may result in different patterns of short-chain fatty acids (SCFAs). Scheppach *et al*. (1988) showed that an induction of starch malabsorption in humans altered the fecal SCFAs pattern in favor of acetate and butyrate. On the other hand, propionate-rich fermentations were promoted in rats fed resistant cornstarch (Morand *et al*., 1992).

Short-chain fatty acids are readily metabolized by man (Hoverstad *et al*., 1982), ruminant (Bergman and Wolff, 1971), and rat (Demigné *et al*., 1986). One result of SCFA production, absorption, and metabolism is the provision of energy to the host. It is estimated that in rats, SCFAs from starch may provide 5−30% of the daily energy intake. SCFAs also have effects on metabolism within the body, specially in the gut mucosa and liver where they are primarily metabolized. Radioisotopes have been extensively used to study volatile fatty acids metabolism in animals (Gaitonde *et al*., 1977; Marty and Vernay, 1984) and in man (Hoverstad *et al*., 1982). In these studies, individual SCFAs were instilled into the caecum and colon. The use of radioactivity in dietary feeding trials includes studies of cellulose (Caryer *et al*., 1982; Walters *et al*., 1989) and ^{14}C plant cell walls (Gray *et al*., 1993; Buchanan *et al*., 1994). The latter work enables the study of fermentation end products collectively as they are produced in the host during bacterial degradation.

The purpose of the present study was to determine the metabolism of the degradation products of U-^{14}C-retrograded bean starch relative to U-^{14}C-gelatinized starch in rat liver and body using an *in vivo* radiolabeled technique.

MATERIALS AND METHODS

Preparation of U-^{14}C-Gelatinized and U-^{14}C-Resistant Bean Starch. Starch was prepared and purified according to the previous description (Abia *et al*., 1996). [U-^{14}C]Starch was obtained from half-filled pods of broad beans incubated under $^{14}CO_2$. After isolation of the starch granules and extraction of lipids and protein, the total starch content in the sample was 94−95% and the specific activity 2.35×10^3 Bq/mg of dry material.

A fraction of 20 mg of [U-^{14}C]-starch (1:4 w/v in distilled water) was gelatinized by autoclaving at 121 °C for 25 min, cooled to ∼30 °C, and immediately fed to rats. A second fraction was retrograded by autoclaving followed by cooling at 6 °C overnight. After five cycles of autoclaving/cooling, (Sievert and Pomeranz, 1989), the samples were freeze-dried, finely ground, and fed to rats. The specific radioactivity in the retrograded samples were 2.17×10^3 Bq/mg of dry material.

Animal Experiments. Animals experiments were performed according to the Home Office Guidelines, U.K. Male

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Wistar Hans rats previously fed a CRM(x) diet with a high starch content were gavaged with 20 mg of either U-^{14}C-gelatinized or [U-^{14}C]-resistant bean starch (8 rats per group). The animals were housed individually in metabolic cages with a broad-spaced grid to minimize coprophagy and were allowed free access to food and water. After 3 and 18 h of feeding, rats were killed by cervical dislocation and immediately dissected (Abia *et al*., 1996).

The carcass (defined as muscle and bone), homogenized in a Waring blender, and the liver were used to study the metabolic fate of [^{14}C]starch degradation products. Approximately 100 mg of fresh tissue was solubilized in sealed vials using 1 mL of Optisolve (Pharmacia Wallac) at 50 °C overnight. After cooling, 10 mL of Hionic-Fluor (Canberra Packard, Berkshire, U.K.) was added and the total activity was measured by liquid scintillation counting. The remaining material was immediately frozen at −70 °C, freeze-dried, and homogenized into a fine powder for further analysis.

Extraction of Lipids. Lipids were extracted in liver and carcass by a modified Folch method (Christie, 1982). A portion of 300 mg of dried sample was mixed with 7 mL of MeOH, left for 30 min, and shaken for a further 5 min. After 14 mL of $CHCl_3$ was added, the samples were shaken for another 5 min and filtered through filter paper (Whatman No. 1). The insoluble phase was rinsed with 9 mL of $CHCl_3$/MeOH (2:1). The $CHCl_3$/MeOH fractions were pooled. The insoluble fraction was dried at room temperature and a portion of 30 mg was solubilized in 1 mL of Optisolve at 50 °C overnight and analyzed for radioactivity in 10 mL of Hionic-Fluor. The remaining sample was stored at −70 °C for further glycogen and protein analysis.

The chloroform/MeOH fraction was washed with 7.5 mL of KCl (0.88% w/v) and the two phases separated. The chloroform fraction was dried using a rotatory evaporator and resuspended in 4 mL of chloroform/methanol (2:1). A portion of 100 μL was dried, redissolved in 500 μL of MeOH, and assayed for radioactivity by adding 5 mL of Pico-Aqua (Canberra Packard, Berkshire, U.K.). The aqueous potassium chloride fraction was dried and redissolved in 5 mL of distilled water and a 1 mL aliquot assayed for ^{14}C using 10 mL of Pico-Aqua.

Separation of Lipids by TLC. Lipids were identified by TLC using silica gel (250 μm) Merck plates 5721. An aliquot of 500 μL of the lipid fraction was spotted as a streak onto TLC plates with a reference mixture of lipids for comparison. The plate was run for 60 min in hexane/diethyl ether/formic acid (80:20:2 by volume).

Plates were dried at room temperature and sprayed with PPO/POPOP (0.6 g/1.0 g in 200 mL of $CHCl_3$) and lipid was identified under UV light.

Bands were scraped into separate tubes and lipids eluted individually from silica gel using 2 × 4 mL of MeOH followed by 3 × 4 mL of $CHCl_3$/MeOH (2:1) (v/v). Samples were evaporated to dryness and redissolved in 500 μL of methanol. Radioactivity was measured by adding 5 mL of Pico-Aqua and counted in a liquid scintillation counter.

Determination of [^{14}C]Glycogen. A portion of the insoluble fraction (150 mg) was digested in 40% (w/v) aqueous potassium hydroxide (5 mL) for 60 min on a boiling water bath (Evans *et al*., 1976). After cooling, two volumes of ethanol were added and the glycogen was left to precipitate overnight at 4 °C. Samples were centrifuged at 1400*g*, and the supernatant was retained for determination of amino acids. The glycogen was purified by three consecutive precipitations with ethanol and centrifugations and redissolved in 1 mL of distilled water; the radioactivity was measured by adding 10 mL of Pico-Aqua.

Determination of [^{14}C]Protein. The protein fraction was neutralized with ∼5 mL of 6.06 M $HClO_4$ and centrifuged at 1400*g* for 10 min. The supernatant was dried in a rotary evaporator and the hydrolysis completed with 6 M HCl containing 10 mM phenol at 110 °C for 24 h. Samples were re-dried and redissolved in 2 mL of 2 M NH_4OH. After neutralization with ∼2 mL of 2 M NaOH, samples were applied to an acid-washed (HCl 1 M) Dowex 50W cation exchange column (volume 15 mL). The column was washed with five column volumes of distilled water to remove unbound material (sugars, Cl^-) (noncationic fraction). Amino acids were eluted using 5 M NH_4OH (four column volumes) (amino acid fraction). Samples were dried, redissolved in distilled water, and stored for further identification. A 500 μL aliquot was measured by liquid scintillation counting by adding 5 mL of Hionic-Fluor.

Table 1. Distribution of ^{14}C (% Bq Gavaged) in the Liver in Rats Fed U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
Folch fractions				
aqueous	$0.82 \pm 0.08^{a,\alpha}$	$0.04 \pm 0.01^{b,\gamma}$	$1.22 \pm 0.10^{c,\beta}$	$0.55 \pm 0.03^{d,\delta}$
insoluble	$0.98 \pm 0.01^{a,\alpha}$	$0.66 \pm 0.05^{b,\gamma}$	$1.21 \pm 0.10^{c,\alpha}$	$1.53 \pm 0.17^{c,\delta}$
lipid	$0.43 \pm 0.06^{a,\alpha}$	$0.25 \pm 0.02^{b,\gamma}$	$0.56 \pm 0.07^{c,\alpha}$	$0.52 \pm 0.05^{c,\delta}$
total	$2.23 \pm 0.8^{a,\alpha}$	$0.95 \pm 0.07^{b,\gamma}$	$2.99 \pm 0.07^{c,\alpha}$	$2.60 \pm 0.23^{c,\delta}$

[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 ($p < 0.05$).

Table 2. Distribution of ^{14}C [Bq(g Wet Wt of Liver)$^{-1}$] in the Liver in Rats Fed U-^{14}C-Gelatinized or (U-^{14}C)-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
Folch fractions				
aqueous	$32 \pm 3^{a,\alpha}$	$1 \pm 0^{b,\gamma}$	$43 \pm 6^{c,\alpha}$	$6 \pm 1^{d,\delta}$
insoluble	$38 \pm 1^{a,\alpha}$	$25 \pm 2^{b,\gamma}$	$50 \pm 4^{c,\alpha}$	$61 \pm 7^{c,\delta}$
lipid	$17 \pm 2^{a,\alpha}$	$9 \pm 1^{b,\gamma}$	$23 \pm 3^{c,\alpha}$	$21 \pm 2^{c,\delta}$

[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.

Identification of [^{14}C]Amino Acids. Amino acids were separated and identified in rat liver 18 h postgavage by using paper chromatography and high-voltage paper electrophoresis. The sample was loaded onto Whatman 3MM paper and developed by ascending chromatography in ethyl acetate/acetic acid/water (10:5:6) for 24 h. Strips cut from the chromatogram were eluted in 5 M NH_4OH; the eluates were dried individually onto paper and assayed for radioactivity by adding 3 mL of scintillation fluid. Strips containing the highest radioactivity were recovered from the scintillation fluid, washed in toluene, dried, and eluted in 5 M NH_4OH. Samples were pooled into three groups and rechromatographed one-dimensionally for 24 h in butanol/acetic acid/water (12:3:5). Samples were treated as before and rechromatographed in butanol/pyridine/water (4:3:4). ^{14}C was determined as above (Fry, 1988).

Aspartic and glutamic acids could not be separated by these solvents and were identified by high-voltage paper electrophoresis in pH 3.5 buffer (pyridine/acetic acid/water 1:10:189) at 240 mA for 45 min. The amino acids used as standards (Sigma Chemical) were stained with 0.5% ninhydrin in acetone.

Assay of ^{14}C. Solutions were measured for 20 min in a Tri-Carb 4430 liquid scintillation counter. The counts were corrected for quenching (external standard). Strips of chromatography and electrophoresis paper were assayed for radioactivity by adding 3 mL of scintillation fluid (PPO (5 g/L) and POPOP (0.5 g/L) in toluene) and counted for 5 min. Results were calculated as the average of three replicates.

Statistical Analysis. Results are given as means ± SEM. Differences between 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.

Table 3. Distribution of ^{14}C (% Bq Gavaged) in the Carcass in Rats Fed U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
Folch fractions				
aqueous	$3.70 \pm 0.20^{a,\alpha}$	$0.82 \pm 0.05^{b,\gamma}$	$4.74 \pm 0.10^{c,\beta}$	$1.83 \pm 0.08^{d,\delta}$
insoluble	$5.81 \pm 0.57^{a,\alpha}$	$3.82 \pm 0.10^{b,\gamma}$	$6.08 \pm 0.20^{c,\alpha}$	$6.38 \pm 0.13^{c,\delta}$
lipid	$1.64 \pm 0.14^{a,\alpha}$	$1.80 \pm 0.09^{a,\gamma}$	$1.91 \pm 0.02^{c,\alpha}$	$2.13 \pm 0.24^{c,\gamma}$
total	$11.15 \pm 0.92^{a,\alpha}$	$6.44 \pm 0.10^{b,\gamma}$	$12.73 \pm 0.30^{c,\alpha}$	$10.34 \pm 0.28^{c,\delta}$

[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 ($p < 0.05$).

Table 4. Distribution of ^{14}C [Bq(g Wet Wt of Carcass)$^{-1}$] in the Carcass in Rats Fed U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
Folch fractions				
aqueous	$16 \pm 1^{a,\alpha}$	$3 \pm 1^{b,\gamma}$	$21 \pm 1^{c,\beta}$	$8 \pm 1^{d,\delta}$
insoluble	$24 \pm 2^{a,\alpha}$	$15 \pm 1^{b,\gamma}$	$36 \pm 5^{c,\alpha}$	$28 \pm 1^{c,\delta}$
lipid	$7 \pm 1^{a,\alpha}$	$7 \pm 1^{a,\gamma}$	$8 \pm 1^{c,\alpha}$	$9 \pm 1^{c,\gamma}$

[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 Mann−Whitney test.

Table 5. Distribution of ^{14}C (% Bq Gavaged) in Glycogen, Sugars, and Amino Acids in Rat Liver after Gavage with U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
glycogen	$0.15 \pm 0.01^{a,\alpha}$	$0.08 \pm 0.01^{b,\gamma}$	$0.17 \pm 0.01^{c,\alpha}$	$0.07 \pm 0.01^{d,\gamma}$
noncation[b]	$0.20 \pm 0.01^{a,\alpha}$	$0.14 \pm 0.01^{a,\gamma}$	$0.37 \pm 0.01^{c,\beta}$	$0.34 \pm 0.05^{c,\delta}$
amino acids	$0.57 \pm 0.07^{a,\alpha}$	$0.38 \pm 0.01^{b,\gamma}$	$0.63 \pm 0.14^{c,\alpha}$	$1.01 \pm 0.06^{c,\delta}$
total	$0.92 \pm 0.06^{a,\alpha}$	$0.60 \pm 0.01^{a,\gamma}$	$1.17 \pm 0.10^{b,\beta}$	$1.42 \pm 0.12^{b,\delta}$

[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 ($p < 0.05$). [b] Sugars, phosphates, organic acids, etc.

Table 6. Distribution of ^{14}C [Bq(g Wet Wt of Liver)$^{-1}$] in Glycogen, Sugars, and Amino Acids in Rat Liver after Gavage with U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
glycogen	$6 \pm 1^{a,\alpha}$	$3 \pm 1^{a,\gamma}$	$7 \pm 1^{c,\alpha}$	$3 \pm 1^{d,\gamma}$
noncation[b]	$4 \pm 1^{a,\alpha}$	$2 \pm 0^{b,\gamma}$	$15 \pm 4^{c,\alpha}$	$10 \pm 2^{c,\delta}$
amino acids	$19 \pm 3^{a,\alpha}$	$10 \pm 1^{b,\gamma}$	$26 \pm 6^{c,\alpha}$	$36 \pm 3^{c,\delta}$

[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. [b] Sugars, phosphates, organic acids, etc.

Table 7. Distribution of ^{14}C (% Bq Gavaged) in Glycogen, Sugars, and Amino Acids in Carcass after Gavage with U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
glycogen	$0.63 \pm 0.01^{a,\alpha}$	0.24 ± 0.03^{b}	$0.01 \pm 0.00^{\beta}$	nd[b]
noncation[c]	$2.38 \pm 0.02^{a,\alpha}$	$1.45 \pm 0.13^{b,\gamma}$	$2.01 \pm 0.31^{c,\alpha}$	$1.58 \pm 0.08^{c,\gamma}$
amino acids	$2.59 \pm 0.10^{a,\alpha}$	$1.91 \pm 0.10^{b,\gamma}$	$4.64 \pm 0.19^{c,\beta}$	$4.65 \pm 0.12^{c,\delta}$
total	$5.60 \pm 0.06^{a,\alpha}$	$3.60 \pm 0.24^{a,\gamma}$	$6.66 \pm 0.24^{b,\beta}$	$6.23 \pm 0.18^{b,\delta}$

[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 ($p < 0.05$). [b] nd, not detected. [c] Sugars, phosphates, organic acids, etc.

RESULTS AND DISCUSSION

The metabolic transformations of ^{14}C metabolites produced during degradation of U-^{14}C-resistant bean starch have been studied in the liver and carcass by comparison with readily digestible U-^{14}C-gelatinized bean starch. Recent studies on rats fed U-^{14}C-resistant starch (Abia *et al.*, 1996) have shown that most of the ^{14}C metabolized by rat tissues was diverted toward carcass (mainly muscle), pelt, and liver. The liver and carcass were therefore chosen for further investigation.

Gelatinized starch will result in the absorption of [^{14}C]glucose from the small intestine; in contrast, ^{14}C metabolites of resistant starch fermentation will be absorbed from the caecum. This implies different substrates being presented to the rat body tissues and therefore the assumed metabolic pathway for ^{14}C degradation products will be different for gelatinized and resistant starch.

In the rat, SCFAs are absorbed from the cecum into the portal vein. In physiological conditions, propionate and butyrate utilization by the liver is highly efficient, whereas acetate uptake is generally limited and depends on the portal concentrations and physiologic conditions. Liver uptake of acetate occurs only when portal blood concentrations are higher than 0.2 mM, (Buckley and Williamson, 1977; Demigné *et al.*, 1986); consequently, acetic acid has been found the only significant fatty acid present in peripheral blood and available to peripheral tissues (Illman *et al.*, 1982).

The distribution of ^{14}C in the aqueous, insoluble, and lipid fractions in the liver and carcass after Folch extraction 3 and 18 h after gavage with U-^{14}C-gelatinized or U-^{14}C-resistant starch is summarized in Tables 1−4. Tissues from rats fed resistant starch had the highest incorporation of radioactivity at all times (Tables 1−4). Liver and carcass incorporated ^{14}C to a different extent. On a per gram of tissue basis, the ^{14}C content was higher in the liver than in carcass (Tables 2 and 4). However, since the carcass is bigger, the total accumulation of radioactivity was larger than in the liver (Tables 1 and 3).

The substitution of U-^{14}C-gelatinized with U-^{14}C-resistant starch showed a significant increase in ^{14}C in the aqueous fraction 3 h after gavage and in all fractions

Table 8. Distribution of ^{14}C [Bq(g Wet Wt of Carcass)$^{-1}$] in Glycogen, Sugars, and Amino Acids in Carcass after Gavage with U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
glycogen	$3 \pm 1^{a,\alpha}$	$1 \pm 0^{b,\gamma}$	nd[b]	nd[b]
noncation[c]	$10 \pm 1^{a,\alpha}$	$6 \pm 1^{b,\gamma}$	$10 \pm 2^{c,\alpha}$	$6 \pm 1^{c,\gamma}$
amino acids	$11 \pm 1^{a,\alpha}$	$8 \pm 1^{b,\gamma}$	$21 \pm 2^{c,\beta}$	$19 \pm 1^{c,\delta}$

[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. [b] nd, not detected. [c] Sugars, phosphates, organic acids, etc.

Table 9. Distribution of ^{14}C (% Bq Gavaged) in Simple Lipid in Rat Liver after Gavage with U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
PL	$0.27 \pm 0.04^{a,\alpha}$	$0.15 \pm 0.01^{a,\gamma}$	$0.31 \pm 0.02^{c,\alpha}$	$0.30 \pm 0.02^{c,\delta}$
MG/DG	$0.01 \pm 0.00^{a,\alpha}$	$0.00 \pm 0.00^{b,\gamma}$	$0.01 \pm 0.00^{c,\alpha}$	$0.01 \pm 0.00^{c,\gamma}$
C	$0.02 \pm 0.00^{a,\alpha}$	$0.01 \pm 0.00^{b,\gamma}$	$0.02 \pm 0.00^{c,\alpha}$	$0.04 \pm 0.00^{d,\delta}$
FFA	$0.01 \pm 0.00^{a,\alpha}$	$0.01 \pm 0.00^{a,\gamma}$	$0.02 \pm 0.01^{c,\alpha}$	$0.01 \pm 0.00^{c,\gamma}$
TG	$0.05 \pm 0.00^{a,\alpha}$	$0.02 \pm 0.00^{b,\gamma}$	$0.06 \pm 0.02^{c,\alpha}$	$0.07 \pm 0.01^{c,\delta}$
CE	$0.01 \pm 0.00^{a,\alpha}$	$0.01 \pm 0.00^{a,\gamma}$	$0.01 \pm 0.00^{c,\alpha}$	$0.01 \pm 0.00^{c,\gamma}$
total	$0.37 \pm 0.04^{a,\alpha}$	$0.20 \pm 0.02^{a,\gamma}$	$0.43 \pm 0.01^{c,\alpha}$	$0.44 \pm 0.01^{c,\delta}$

[a] Lipids: PL, phospholipids, MG/DC, monoacylglycerols/diacylglycerols; C, cholesterol; FFA, free fatty acids; TG, triacylglycerols; CE, cholesterol esters. 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 ($p < 0.05$).

after 18 h of feeding in both the liver and carcass (Tables 1 and 3). The high radioactivity present in the aqueous fraction after 3 h of feeding could be attributed to the production, absorption, and metabolism of ^{14}C end products of fermentation. Studies on the metabolism of [^{14}C]SCFAs in rabbit liver have shown high radioactivity incorporated into products of intermediary metabolism such as free amino acids and citric cycle acids such as succinate, malate, and sugars (Marty and Vernay, 1984). The radioactivity in the aqueous fraction decreased markedly from 3 to 18 h in both liver and carcass, possibly due to oxidative metabolism or the synthesis of body components such as proteins. The distribution of ^{14}C in the insoluble fraction [glycogen, noncationic compounds (sugars, phosphates, organic acids, etc), and proteins] in liver and carcass (Tables 5−8) showed that, at 18 h, the radioactivity was mostly channeled toward the incorporation into the nonglycogen fraction and specially the protein one (Tables 5 and 7).

The identification of labeled amino acids released from proteins of the liver by paper chromatography showed that ^{14}C was predominantly associated with aspartate/glutamate and smaller amounts of glycine/serine (65% of the total radioactivity) (Figure 1A), alanine (28%) (Figure 1B), and to a lesser extent alanine along with proline and hydroxyproline (6%) (Figure 1C).

The conversion of propionate into free aspartate, glutamate, and alanine has been demonstrated in rat liver (Gaitonde *et al.*, 1977; Desmoulin *et al.*, 1985). Propionate was suggested to contribute to the formation of free amino acids by entering the citric acid as intramitochondrial succinyl-CoA. The succinate is oxi-

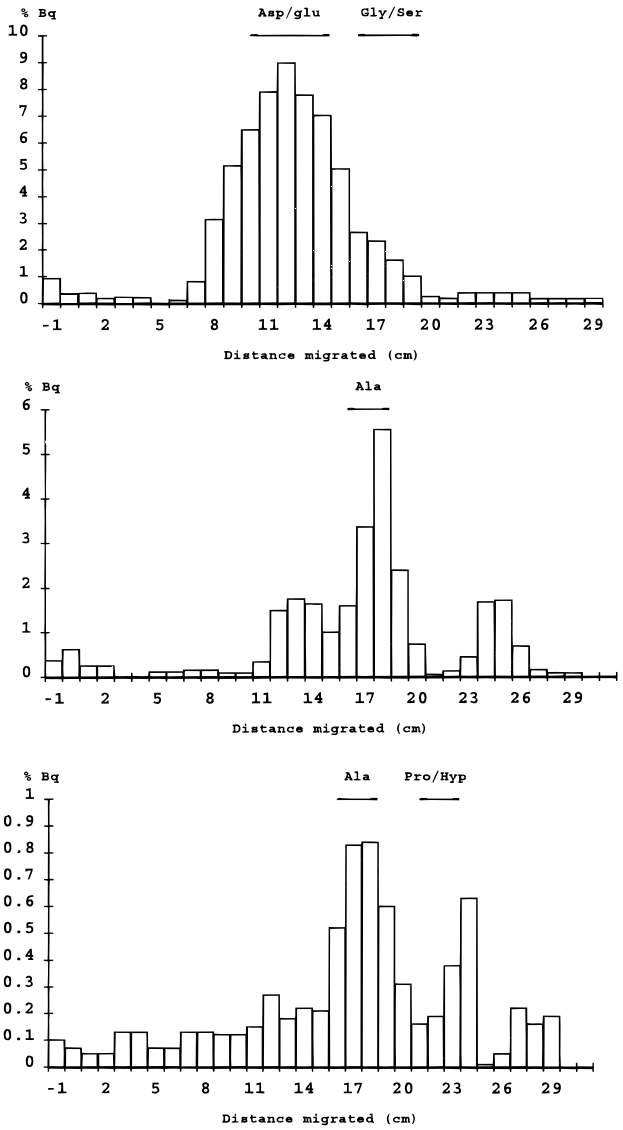

Figure 1. Paper chromatography in butanol/pyridine/water (4:3:4 by volume) of rat liver amino acids 18 h postgavage. Markers: Asp, aspartic acid; Glu, glutamic acid; Gly; glycine; Ser; serine, Ala; alanine, Pro; proline, Hyp; hydroxyproline.

dized to oxaloacetate, which is the precursor of aspartate. Our findings are in keeping with these results and suggest that the disposition of this amino acid in proteins during the postabsorptive period constitutes an important metabolic pathway for degradation products of resistant starch.

It has been reported that diets containing amylomaize starch (starch high in amylose) promote the flux of ammonia from bacterial metabolism to efferent blood. This could influence hepatic protein metabolism, since nearly all portal vein ammonia is taken up by the liver. Volatile fatty acids have also been suggested to contribute to the sparing of protein due to a mobilization of amino acids for gluconeogenesis during the postabsorptive period (Rémésy and Demigné, 1989).

The ^{14}C incorporated into hepatic glycogen did not differ between rats fed gelatinized starch and resistant starch, and in both cases a decrease in radioactivity was observed from 3 to 18 h (Tables 5 and 6). Resistant starch has been found to have a glycogenogenic effect, possibly due to the production of propionic acid during fermentation (Morand *et al.*, 1992). The ^{14}C incorporated into glycogen in the carcass of rats fed resistant starch was minor and not detected after 18 h of feeding

Table 10. Distribution of ^{14}C [Bq (kg Wet Wt of Liver)$^{-1}$] in Simple Lipid in Rat Liver after Gavage with U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
PL	$8326 \pm 1592^{a,\alpha}$	$5128 \pm 1134^{a,\gamma}$	$10758 \pm 1886^{c,\alpha}$	$10717 \pm 1038^{c,\delta}$
MG/DG	$328 \pm 24^{a,\alpha}$	$128 \pm 20^{b,\gamma}$	$575 \pm 210^{c,\alpha}$	$238 \pm 44^{c,\gamma}$
C	$628 \pm 15^{a,\alpha}$	$406 \pm 64^{a,\gamma}$	$717 \pm 69^{c,\alpha}$	$1738 \pm 88^{d,\delta}$
FFA	$128 \pm 24^{a,\alpha}$	$72 \pm 29^{b,\gamma}$	$775 \pm 648^{c,\alpha}$	$462 \pm 285^{c,\delta}$
TG	$1778 \pm 159^{a,\alpha}$	$739 \pm 36^{b,\gamma}$	$2463 \pm 648^{c,\alpha}$	$2688 \pm 300^{c,\delta}$
CE	$133 \pm 29^{a,\alpha}$	$111 \pm 34^{a,\gamma}$	$513 \pm 347^{c,\alpha}$	$346 \pm 46^{c,\delta}$

[a] Lipids: PL, phospholipids, MG/DC, monoacylglycerols/diacylglycerols; C, cholesterol; FFA, free fatty acids; TG, triacylglycerols; CE, cholesterol esters. 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 11. Distribution of ^{14}C (% Bq Gavaged) in Simple Lipids in Carcass after Gavage with U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
PL	$0.49 \pm 0.04^{a,\alpha}$	$0.30 \pm 0.01^{b,\gamma}$	$0.58 \pm 0.01^{c,\alpha}$	$0.39 \pm 0.01^{d,\gamma}$
MG/DG	$0.06 \pm 0.01^{a,\alpha}$	$0.07 \pm 0.01^{a,\gamma}$	$0.12 \pm 0.01^{c,\alpha}$	$0.08 \pm 0.00^{c,\gamma}$
C	$0.07 \pm 0.01^{a,\alpha}$	$0.06 \pm 0.01^{a,\gamma}$	$0.03 \pm 0.01^{c,\alpha}$	$0.14 \pm 0.00^{d,\delta}$
FFA	$0.22 \pm 0.05^{a,\alpha}$	$0.17 \pm 0.01^{a,\gamma}$	$0.28 \pm 0.01^{c,\alpha}$	$0.30 \pm 0.02^{c,\delta}$
TG	$0.58 \pm 0.03^{a,\alpha}$	$0.75 \pm 0.01^{b,\gamma}$	$0.56 \pm 0.02^{c,\alpha}$	$0.93 \pm 0.23^{c,\gamma}$
CE	$0.13 \pm 0.01^{a,\alpha}$	$0.15 \pm 0.00^{a,\gamma}$	$0.17 \pm 0.02^{c,\alpha}$	$0.22 \pm 0.02^{c,\gamma}$
total	$1.55 \pm 0.05^{a,\alpha}$	$1.50 \pm 0.01^{a,\gamma}$	$1.74 \pm 0.07^{c,\alpha}$	$2.06 \pm 0.24^{c,\gamma}$

[a] Lipids: PL; phospholipids, MG/DG, monoacylglycerols/diacylglycerols; C, cholesterol; FFA, free fatty acids; TG, triacylglycerols; CE, cholesterol esters. 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 12. Distribution of ^{14}C [Bq (kg Wet Wt of Carcass)$^{-1}$] in Simple Lipids in Carcass after Gavage with U-^{14}C-Gelatinized or U-^{14}C-Resistant Bean Starch[a]

	radioactivity derived from [^{14}C]starch			
	gelatinized		resistant	
	3 h	18 h	3 h	18 h
PL	$2062 \pm 173^{a,\alpha}$	$1222 \pm 29^{b,\gamma}$	$2539 \pm 49^{c,\alpha}$	$1739 \pm 20^{d,\gamma}$
MG/DG	$272 \pm 46^{a,\alpha}$	$306 \pm 24^{a,\gamma}$	$507 \pm 54^{c,\alpha}$	$361 \pm 20^{c,\gamma}$
C	$289 \pm 20^{a,\alpha}$	$256 \pm 24^{a,\gamma}$	$133 \pm 13^{c,\beta}$	$594 \pm 15^{d,\delta}$
FFA	$928 \pm 192^{a,\alpha}$	$722 \pm 34^{a,\gamma}$	$1233 \pm 35^{c,\alpha}$	$1322 \pm 78^{c,\delta}$
TG	$2467 \pm 56^{a,\alpha}$	$3083 \pm 73^{b,\gamma}$	$2472 \pm 101^{c,\alpha}$	$4117 \pm 997^{c,\gamma}$
CE	$539 \pm 56^{a,\alpha}$	$600 \pm 19^{a,\gamma}$	$733 \pm 96^{c,\alpha}$	$983 \pm 92^{c,\gamma}$

[a] Lipids: PL; phospholipids, MG/DG, monoacylglycerols/diacylglycerols; C, cholesterol; FFA, free fatty acids; TG, triacylglycerols; CE, cholesterol esters. 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 7), which may indicate the lack of glycogenogenic precursors available to extrasplacnic tissues.

The substitution of the readily digestible starch with a starch resistant to pancreatic digestion increased the incorporation of ^{14}C in the liver total lipid level 18 h postgavage (Table 1). Separation of lipids by TLC (Tables 9 and 10) showed that the metabolism of resistant starch degradation products led to a significant increase in the radioactivity in phospholipids, cholesterol, and triacylglycerols. The specific radioactivity in free fatty acids and cholesterol esters was also higher in rats fed label resistant starch (Table 10). There is evidence that acetic and butyric acids may be incorporated into free fatty acids, cholesterol, triglycerides, and phospholipids (Arce *et al.*, 1993; Snoswell *et al.*, 1982; Latymer and Woodley, 1983). Although no significant changes in total labeled lipids were detected in the carcass (Table 3), ^{14}C increased significantly in the cholesterol and free fatty acid fractions 18 h after gavage in rats fed resistant starch compared to rats fed gelatinized starch (Tables 11 and 12). Recent studies using ^{14}C-labeled spinach cell walls (Buchanan *et al.*, 1994) have shown that the metabolism of the ^{14}C-labeled fermentation end products by the liver resulted in the labeling of phospholipids and triacylglycerols, but not cholesterol. This results compared to our findings indicates that the incorporation of ^{14}C into lipids seems to depend on the kind of substrate fermented and, consequently, on the proportions of the final products taken up by the liver.

In summary, the substitution of a [U-^{14}C]starch easily digested in the small intestine with [U-^{14}C]starch resistant to amylolytic digestion provided the body with ^{14}C which appeared to be predominantly incorporated into amino acids (mainly glutamic/aspartic acids and alanine). Less incorporation of ^{14}C was detected in lipids, being mainly accumulated in phospholipids, cholesterol, and triacylglycerols in liver and cholesterol and free fatty acids in carcass.

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