Effect of Maillard Reaction Product on Bile Acid Binding, Plasma and Hepatic Lipids, and Weight of Gastrointestinal Organs

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The objective was to determine the bile acid binding capacity of Maillard reaction products (MRPs) and its effect on plasma cholesterol (chol) and gastrointestinal weight in rats. MRP was prepared by heating a mixture of 3 M α-D-glucose and 3 M L-lysine at pH 9.0 and washing with ethanol to reduce low molecular weight MRP. In vitro data indicated that high molecular weight MRP bound cholic and chenodeoxycholic acid. Purified diets were prepared with 5% cellulose (C), 2.5% MRP plus 2.5% cellulose (CM), or 5% MRP (M) and fed to three groups of male Wistar rats ($n = 10$) for 4 weeks. Each diet contained 1% chol and 0.2% cholic acid to induce hypercholesterolemia. In the M group plasma chol was significantly higher than in the C group, but liver chol did not differ between the C and M groups. In the CM group liver chol was higher but plasma chol did not differ from that in the C group. Total small intestine and mucosa weights were significantly higher in the M than in the C or CM groups. The cecum in the M group was about 3-fold heavier than in the C or CM groups. Thus, MRPs do not lower chol levels; however, they do increase gastrointestinal weight.

INTRODUCTION

The Maillard reaction involves the condensation of sugars and amino acids and results in the formation of a wide span of aromas and brown pigments referred to as melanoidins or Maillard reaction products (MRPs). The Maillard reaction is used in many processes such as torrefaction or roasting to develop desirable characteristics in foods. It can become undesirable, however, when it is not controlled and produces poor color and off-flavors. The presence of MRPs alters the nutritive value of protein sources by destroying amino acids and reducing protein digestibility (Mauron, 1981; Adrian, 1974). MRPs seem to interfere with the activity of several digestive enzymes in the rat (Öste et al., 1983, 1985; Percival and Schneeman, 1979). It is believed that the brown products are not only antinutritional but toxic as well. When incorporated into protein-adequate diets, they increase liver and kidney weights, a possible sign of toxicity or nutritional deficiency (O'Brien and Walker, 1988; Pintauro et al., 1983). Moreover, they increase the occurrence of fetal death in pregnant rats (Adrian, 1974) and exhibit some mutagenic effects in food systems such as cooked meats (Barnes et al., 1983).

Despite these negative aspects, MRPs appear to share some physiological properties with dietary fibers and may have fiberlike actions. The high molecular weight MRPs are mostly undigested (Nair et al., 1981; Finot and Magnenat, 1981) and appear to be fermented by the rat microflora (Horikoshi et al., 1981). They have a high water-holding capacity (O'Brien and Walker, 1988), interfere with mineral metabolism because of their high cation-exchange capacity (McBurney et al., 1983), and are capable of binding organic molecules (Horikoshi and Gomyo, 1976).

Pintauro et al. (1983) observed a hypocholesterolemic effect of MRP in the rat and proposed that these products could bind intestinal bile acids and increase bile acid excretion in the feces. To maintain the bile acid pool, the production of bile acids from cholesterol in the liver would be enhanced, thereby lowering plasma cholesterol levels. This phenomenon has been well described for certain dietary fibers. Very little information, however, is available on the possible bile acid binding capacity and hypocholesterolemic effect of MRP. Thus, the objective of this study was to determine the ability of MRPs to bind bile acids in vitro and alter lipid levels in an animal model.

MATERIALS AND METHODS

In Vitro Experiments. *Maillard Reaction Model Systems.* Sixteen grams of α-D-glucose (Sigma, St. Louis, MO) was mixed with either 84 g of egg albumen (ICN Pharmaceuticals, Cleveland, OH) or casein (Dyets, Bethlehem, PA). The mixtures were heated at 121 °C in an autoclave for 24 h. These two systems were called EA and CA, respectively. Equal volumes of a 1 M solution of α-D-glucose and a 1 M solution of L-lysine (Sigma) were mixed. This mixture was heated at 121 °C in an autoclave for 1 h, cooled, and freeze-dried for 42 h. The resulting Maillard reaction system was called L1. In system L2, 3 M solutions of α-D-glucose and L-lysine were mixed to form a solution whose pH was adjusted to 9.0 with 10 N NaOH to enhance the intensity of the reaction. This solution was heated at 121 °C in an autoclave for 3 h and was adjusted to pH 7.0 after cooling. Absolute ethanol was added to a concentration of 90% by volume. The resulting suspension was centrifuged at 23000*g* and 4 °C for 30 min, and the supernatant was discarded. This centrifugation step was conducted to eliminate low molecular weight MRP. The precipitated MRP fraction was dispersed in distilled water, residual alcohol evaporated, and then the fraction lyophilized. Thus, four model systems containing MRP were tested in vitro: EA (egg albumin plus glucose), CA (casein plus glucose), L1 (lysine plus glucose), and L2 (lysine plus glucose treated to enhance formation of high molecular weight MRP).

Bile Acid Binding Assay. The bile acid adsorption capacity of each Maillard reaction system was measured using a variation of the method of Story and Kritchevsky (1976). Solutions of sodium–cholic acid (Sigma) and sodium–chenodeoxycholic acid (Calbiochem, La Jolla, CA) were prepared in Hepes buffer (pH 8.0) at a concentration of 50 mM. Labeled bile acids, [^{3}H]cholic acid (Du Pont Co., NEN Research Products, Boston, MA) or [^{14}C]chenodeoxycholic acid (Du Pont, NEN Research Products), were added to these solutions in the following proportions: 2 μCi of [^{3}H]cholic acid/mL of sodium–cholic acid solution and 0.5 μCi of [^{14}C]chenodeoxycholic acid/mL of sodium chenodeoxycholic acid solution. Triplicate samples (50–100 mg) of each Maillard

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reaction system were incubated with 0.5 mL of the bile acid solution and 4.5 mL of Hepes buffer in screw-cap culture tubes and shaken in a water bath at 37 °C for 3 h. A half milliliter of each supernatant was transferred into a filter unit with a molecular weight cutoff of 30 000 (Centrifree, Amicon, Danvers, MA) that was centrifuged at 2000*g* and 25 °C for 1 h. Finally, 100 μL of the filtrate was assayed for radioactivity using a Tri-Carb 2660 liquid scintillation system (Packard, Laguna Hills, CA). Filtrate activity of the samples is a measure of unbound bile acids, whereas filtrate activity of blanks (no MRP present) is a measure of total bile acids. The bound bile acids were calculated by subtracting these two values. The results were expressed as the percentage of bile acids that are bound.

The bile acid adsorption capacity of the various molecular weight fractions of the Maillard reaction system L2 were also assayed. The amount of MRP tested in this experiment was 75 mg instead of 50 or 100 mg. Also, to characterize binding by molecular weight, filter units (Amicon) with cutoffs of 3000, 10 000, or 30 000 were used.

Statistical Analysis. Statistical analysis was by one-way analysis of variance with Dunnett test to compare each MRP system with the blank, using Statview 512+ (version 1.1, Abacus Concepts, Inc.). Adsorption capacities are expressed as mean ± SEM. Values with an asterisk are significantly different from the blank ($p < 0.05$).

In Vivo Experiments. *Preparation and Characterization of MRP for Animal Diets.* MRP (600 g) was made as described above (system L2) using 1 L of 3 M α-D-glucose solution and 1 L of 3 M L-lysine solution. The free glucose content of the MRP was measured by the glucose oxidase assay with a Beckman glucose analyzer 2 (Beckman, Fullerton, CA). To determine the lysine content of the MRP, a 0.73 g/L solution was adjusted to pH 2.2. A 50-mL aliquot of the solution was loaded on the ion-exchange column of a Beckman 6300 amino acid analyzer (Beckman) equilibrated with a 0.2 M lithium citrate solvent. The column outflow was monitored at 570 nm.

The molecular weight distribution of the MRP was determined by gel chromatography using a 1.6 × 60 cm chromatography column (LKB, Bromma, Sweden) equipped with a flow adapter and a sample applicator. Its outlet was connected to a LKB 2120 Varioperpex II pump (LKB). This pump was coupled to a LKB 2112 Redirac fraction collector (LKB). The column was packed with Sephadex G-50 (Sigma) previously swollen in a 2 M NaCl solution. The void volume was determined from the elution volume of a 0.2% blue dextran 2000 (Pharmacia Fine Chemicals, Uppsala, Sweden) solution (in 2 M NaCl), whereas the total bed volume was estimated from the column dimensions. One gram of MRP was dissolved in 6 mL of degassed 2 M NaCl and applied to the column. The eluant was 2 M NaCl, and the flow rate was 19 mL/h. One hundred fractions were collected, and their absorbance was measured at the maximum absorbance wavelength of MRP (300 nm) using a Shimadzu UV–vis recording spectrophotometer (Shimadzu, Kyoto, Japan). Fractions 24–33, 34–51, 52–64, 81–90, and 91–100 were combined into pools and numbered 1, 2, 3, 4, and 5, respectively. Each fraction was quantitatively transferred to a calibrated buret to determine the total pool volume. Each pool was lyophilized, placed overnight in a desiccator (filled with fresh calcium sulfate), and weighed. As no MRP was present in pools 4 and 5, they were used to estimate the NaCl concentration of the eluted fractions and correct for the weight of NaCl present in pools 1–3. On the basis of the total weight of MRP and the weight of MRP present in pools 1–3, the percentage weight of diet MRP of each pool was estimated. Pools 1, 2, and 3 correspond to the following molecular weight fractions: greater than 10 000, 500–10 000, and less than 500, respectively.

Experimental Diets and Protocol. Three purified diets were fed: a control diet that contained 5% cellulose (C), a diet containing 2.5% cellulose and 2.5% MRP (CM), and a diet containing 5% MRP (M). The composition of the diets is shown in Table I.

Thirty male Wistar rats (Hilltop Laboratories, Scottsdale, PA) with an initial average body weight of 134 ± 5 g (SD) were individually housed in wire-bottomed cages. They were kept in a temperature- (21–23 °C) and light-controlled room with a 12-h light–dark cycle beginning at 7:00 a.m. The animals were fed ad libitum a stock diet (Rat Chow, Purina Mills, St. Louis, MO) for 7 days, after which time they were randomly assigned to one of the three diets (10 per group) for a 4-week period during which food and water were consumed ad libitum. Food intake was measured during the second week of the study and during week 4. Animals were weighed once a week and immediately before they were killed.

Table I. Composition of Control and Experimental Diets (Grams per 100 g)

ingredient	5% cellulose (C)	2.5% cellulose + 2.5% MRP (CM)	5% MRP (M)
cellulose (Solka Floc)	5.0	2.5	
MRP		2.5	5.0
casein	22.0	22.0	22.0
sucrose	13.0	13.0	13.0
corn oil	10.0	10.0	10.0
cornstarch	43.78	43.78	43.78
mineral mix[a]	3.5	3.5	3.5
vitamin mix[b]	1.0	1.0	1.0
choline chloride	0.2	0.2	0.2
DL-methionine	0.3	0.3	0.3
cholesterol	1.0	1.0	1.0
cholic acid	0.2	0.2	0.2
BHT	0.02	0.02	0.02
total	100.0	100.0	100.0

[a] Salt content of the mineral mix (g/kg): $CaCO_3$ (anhydrous), 238.0; H_2HPO_4, 321.0; $CaHPO_4$ (anhydrous), 60.0; NaCl, 43.0; $FeSO_4 \cdot 7H_2O$, 10.0; $MgSO_4$ (anhydrous, 97.0; KI, 0.20; $ZnCO_3$, 1.60; Na_2SeO_3, 0.0018; $CuSO_4 \cdot 5H_2O$, 0.66; $CrK(SO_4)_2 \cdot 12H_2O$, 0.40; $MnSO_4 \cdot H_2O$, 2.30. [b] Vitamin composition per kg of vitamin mix (g): choline chloride (70% solution), 71.5 mL; inositol, 25.0; ascorbic acid, 5.0; calcium pantothenate, 2.5; thiamin hydrochloride, 1.5; pyridoxine, 1.5; nicotinic acid, 1.5; menadione, 1.25; riboflavin, 0.5; *p*-aminobenzoic acid, 0.5; folic acid, 0.03; biotin, 0.0125; Rovimix E-50% (500 000 IU/kg), 7.8; Rovimix A-650 (650 000 IU/g), 0.2051; Rovimix AD_3 A650/D325 (650 000 IU A/g, 325 000 IU D_3/g), 0.2051; 0.1% B_{12} in mannitol (ICN), 1.5; cerelose, 879.49.

At the end of the 4-week study, the rats were fasted overnight and injected with a solution of ketamine (50 mg/kg of body weight), rompum (5 mg/kg of body weight), and acepromazine (0.75 mg/kg of body weight). Blood was removed by heart puncture with EDTA (1 mg/mL of blood) as an anticoagulant. The intestinal tract (small intestine, large intestine, and cecum), liver, and pancreas were removed and weighed, and the small intestine was scraped with a glass slide to remove the mucosa. All tissues were frozen.

Plasma was obtained from blood by centrifugation at 1500*g* and 4 °C for 20 min. Lipoproteins were fractionated from 2-mL plasma samples containing 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) by sequential ultracentrifugation, using a Sorvall fixed-angle rotor (TFT 45.6) in a Sorvall OTD-65B ultracentrifuge (Du Pont, Wilmington, DE) at 160000*g*. Lipoprotein classes were separated as described previously (Ney et al., 1986; Lindgren, 1975). Gradient gel electrophoresis was conducted on fresh undialyzed HDL and LDL fractions, and quantification of protein concentrations was done on undialyzed fractions. Cholesterol, triglyceride, and apolipoprotein determinations were performed on dialyzed fractions. Lipids were extracted from 0.5 g of liver by a Folch extraction (Folch et al., 1957).

Analytical Procedures. Plasma, lipoprotein, and hepatic cholesterol levels were determined by the cholesterol oxidase method (Allain et al., 1974). Total cholesterol and free cholesterol were determined with or without cholesterol esterase in the assay mixture, respectively, and cholesterol ester concentration was estimated by difference (Warnick et al., 1982). Plasma and lipoproteins were analyzed for triglycerides by estimating glycerol release (Bucolo and David, 1973; Technical Bulletin 336, Sigma). Liver triglycerides were determined colorimetrically on the liver lipid extract (Sigma Technical Bulletin 405). Lipoproteins were analyzed for total protein using a modified Lowry reagent (Sigma Technical Bulletin P5656).

Apolipoproteins B and AI were estimated by rocket electroimmunoassay following the method of Laurell (1966). Plasma samples and dilutions of standardized rat plasma of known apolipoprotein concentrations were applied to agarose and poly(ethylene glycol) (Apo B) or dextran (Apo AI) gels containing

Table II. Adsorption of Cholic Acid and Chenodeoxycholic Acid by Maillard Reaction Products (MRP)[a-c]

bile acid	Maillard reaction system[d]	bile acid adsorption capacity,[e] % 50 mg of MRP	100 mg of MRP
cholic	blank	0.00 ± 0.56	0.00 ± 0.56
	CA	1.28 ± 1.43	3.91 ± 1.82
	EA	3.03 ± 0.69	9.25 ± 0.70*
	L1	6.29 ± 1.73*	8.67 ± 2.00*
	L2	9.40 ± 0.26*	13.33 ± 1.54*
chenodeoxycholic	blank	0.00 ± 0.65	0.00 ± 0.65
	CA	11.85 ± 4.57*	21.94 ± 0.51*
	EA	11.17 ± 0.19*	25.72 ± 4.33*
	L1	3.92 ± 1.12	8.29 ± 0.78*
	L2	15.26 ± 1.53*	29.27 ± 1.70*

[a] 50 or 100 mg of binding material; 25 μmol of bile acid in 5 mL of Hepes buffer, pH 8. [b] Values are means ± SEM; $n = 3$ replicates. [c] Values with an asterisk differ significantly from the blank ($p < 0.05$). [d] Abbreviations: CA, casein + glucose; EA, egg albumen + glucose; L1, L-lysine + glucose; L2, L-lysine + glucose; MRP, Maillard reaction products. [e] Ratio of bound bile acids to total bile acids × 100.

antibodies to apolipoproteins B and AI. The antisera were obtained from Dr. Paul Roheim (Louisiana State University, New Orleans, LA). The gels were subjected to electrophoresis at 10 °C and 50 (Apo B) or 100 V (Apo AI) for 19.5 h in 14.4 g/L Tricine buffer. They were then rinsed with 0.15 M NaCl, dried, stained with 0.05% Coomassie Brilliant Blue (R-250, Bio-Rad, Richmond, CA) in methanol/acetic acid/water (45:10:45 by volume), and rinsed with distilled water. Concentrations were proportional to peak heights and were estimated from the standard curve.

Gradient gel electrophoresis was used to determine the relative distribution of particles corresponding to the HDL-1 (diameter > 11.8 nm) and HDL-2 (diameter 7.0–11.8 nm) size ranges (Oschry and Eisenberg, 1982) as described previously (Ney et al., 1986; Blanche et al., 1981).

Statistical Analysis. Statistical analysis of the data was by one-way analysis of variance with LSD to compare groups, using Statview 512+ (version 1.1, Abacus Concepts). The correlation between total intestinal weight and dietary MRP was also determined. Values are expressed as mean ± SEM of individual groups. Values with a different subscript letter are significantly different ($p < 0.05$).

RESULTS

Bile Acid Binding Assay. Significant levels of bile acid adsorption were observed with most of the MRP tested (Table II). Additionally, most of the binding capacities doubled when the quantity of MRP in the assay was raised from 50 to 100 mg. The only system that had a strong affinity for both cholic acid and chenodeoxycholic acid was system L2. Protein systems (CA and EA) were almost as efficient as L2 in binding chenodeoxycholic acid but not cholic acid. The two lysine systems L1 and L2 seemed to behave differently. Adsorption of both bile acids was higher with L2 than L1. This difference suggested that the treatment applied to L2, i.e., the removal of low molecular weight compounds from the Maillard reaction mixture, was the method of choice for the preparation of the MRP to be incorporated in the animal diets. Filters with different molecular weight cutoffs were used to analyze three molecular weight fractions of the L2 MRP: >30 000, >10 000, and >3000. The only difference between the >30 000 and the >3000 fractions in the presence of MRPs with molecular weights between 3000 and 30 000 in the latter. As shown in Table III, the presence of molecules with a molecular weight in this range raised the adsorption capacity of the L2 MRP by only about 5%, for both cholic acid and chenodeoxycholic acid, compared to the assay with only the high molecular weight compounds (>30 000). Therefore, it can be concluded that high molecular weight compounds (>30 000) are the ones which contribute to a large extent to the high bile acid binding capacity by the L2 MRP.

Table III. Adsorption of Cholic Acid and Chenodeoxycholic Acid by Maillard Reaction Products[a-c]

bile acid	molecular wt cutoff	bile acid adsorption capacity,[d] % blank	L2[e]
cholic	30 000	0.00 ± 0.88	13.71 ± 0.36*
	10 000	0.00 ± 0.81	14.79 ± 0.61*
	3 000	0.00 ± 0.73	18.58 ± 0.83*
chenodeoxycholic	30 000	0.00 ± 0.57	23.70 ± 1.42*
	10 000	0.00 ± 0.78	29.36 ± 0.68*
	3 000	0.00 ± 0.49	28.23 ± 0.48*

[a] 50 or 100 mg of binding material; 25 mmol of bile acid in 5 mL of Hepes buffer, pH 8. [b] Values are means ± SEM; $n = 3$ replicates. [c] Values with an asterisk differ significantly from the blank ($p < 0.05$). [d] Ratio of bound bile acids to total bile acids × 100. [e] L2, L-Lysine + glucose (high molecular weight compounds).

Table IV. Food Intakes and Body Weights[a-c]

	5% cellulose (C)	2.5% cellulose + 2.5% MRP (CM)	5% MRP (M)
daily food intake, g			
week 2	21.8 ± 0.9[a]	23.8 ± 0.4[b]	20.3 ± 0.4[a]
week 4	19.9 ± 0.8[a]	21.8 ± 0.5[b]	20.3 ± 0.5[ab]
body wt, g			
initial	182 ± 2	184 ± 2	183 ± 2
week 1	237 ± 4[a]	250 ± 2[b]	240 ± 3[a]
week 2	286 ± 6[a]	308 ± 3[b]	287 ± 3[a]
week 3	326 ± 8[a]	355 ± 5[b]	328 ± 4[a]
week 4	342 ± 10[a]	374 ± 7[b]	343 ± 6[a]

[a] Values are means ± SEM; $n = 10$. [b] Values with different superscript letters differ significantly ($p < 0.05$). [c] Body weights were measured at the beginning of the study and at the end of each week of the study.

Characterization of MRP for Experimental Diets. The glucose content of the MRP was 0.95% (w/w), whereas the L-lysine content was 43.7% (w/w). The high L-lysine quantity present in the diet MRP, together with its low glucose content, suggests that the limiting element of the Maillard reaction was the glucose concentration.

Following elution of 1 g of diet MRP by gel chromatography, three pools were made of fractions containing MRP, as indicated by absorbance at 300 nm. Pool 1 contained 28.8% of the material chromatographed and had a molecular weight >10 000; pool 2 contained 24.5% and had a molecular weight between 500 and 10 000; and pool 3 contained 46.7% and had a molecular weight below 500. Pool 3 corresponds to the pool containing the remaining substrates, glucose (0.95%) and L-lysine (43.7%). Pools 1 and 2, which contain the MRP, were 54% of the total weight.

In Vivo Experiments. *Food Intakes and Body Weights.* The food intakes of all animal groups did not change between the second and the last week of the study (Table IV). After 2 weeks, animals fed CM had significantly higher food intakes and body weights than the control group, whereas the animals fed M did not differ from the control group. Previous studies have reported that at low levels MRPs increase food intake, undoubtedly due to their enhancement of the flavor of the diet (Homma and Fujikama, 1981; Andrieux et al., 1980; Adrian, 1974).

Tissue Weights. The weights of intestinal sections, liver, and pancreas are expressed as the weight per 100 g of body weight and are shown in Table V. The intestinal tract weight relative to body weight was higher in the M group than in the control. This effect was due to larger small intestine and cecum weights per 100 g of body weight

Table V. Organ Weights plus Contents (Grams) per 100 g of Body Weight[a,b]

organ	5% cellulose (C)	2.5% cellulose + 2.5% MRP (CM)	5% MRP (M)
small intestine	1.65 ± 0.035[a]	1.69 ± 0.035[a]	1.92 ± 0.057[b]
large intestine	0.304 ± 0.015	0.356 ± 0.028	0.312 ± 0.023
cecum	0.48 ± 0.03[a]	0.58 ± 0.04[a]	1.70 ± 0.12[b]
intestinal tract	2.44 ± 0.06[a]	2.63 ± 0.07[a]	3.93 ± 0.15[b]
liver	5.26 ± 0.15[ab]	5.69 ± 0.15[b]	5.08 ± 0.17[a]
pancreas	0.288 ± 0.012	0.279 ± 0.021	0.275 ± 0.011
mucosa	0.343 ± 0.016[a]	0.391 ± 0.018[a]	0.612 ± 0.049[b]

[a] Values are means ± SEM; numbers in parentheses are the numbers of samples if they differ from the original sample size, n = 10. [b] Values with different superscript letters differ significantly ($p < 0.05$).

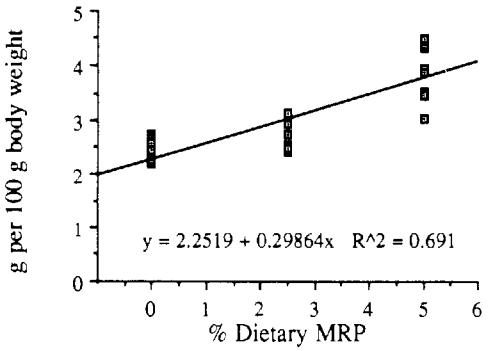

Figure 1. Correlation between level of MRP added to the diet and weight of the intestinal tract (small intestine and large intestine plus cecum) per 100 g of body weight. Correlation coefficient is significant (p = 0.0001, N = 30).

Table VI. Cholesterol and Triglyceride Concentrations in Plasma and Liver[a,b]

	5% cellulose (C)	2.5% cellulose + 2.5% MRP (CM)	5% MRP (M)
plasma (mg/dL)			
cholesterol			
total	106.4 ± 6.5[a]	95.6 ± 5.8[a]	129.2 ± 8.6[b]
free	22.4 ± 1.7[a]	20.4 ± 1.5[a]	27.8 ± 1.8[b]
esterified	84 ± 5.0[a]	75.2 ± 4.6[a]	101.4 ± 6.8[b]
free/esterified	0.27 ± 0.01	0.27 ± 0.01	0.28 ± 0.01
triglycerides	81.3 ± 9.0	72.9 ± 2.7	85.4 ± 8.4
apolipoprotein AI	64.9 ± 3.7	64.3 ± 6.2	66.7 ± 5.7 (9)
apolipoprotein B	72.6 ± 4.5	75.1 ± 9.8	62.2 ± 3.5 (9)
liver (mg/g)			
cholesterol			
total	44.7 ± 2.8[a]	56.8 ± 2.6[b]	47.2 ± 3.0[a]
free	3.3 ± 0.1[a]	3.9 ± 0.1[b]	3.8 ± 0.2[b]
esterified	41.4 ± 2.7[a]	52.9 ± 2.6[b]	43.4 ± 2.9[a]
free/esterified	0.08 ± 0.01	0.08 ± 0.00	0.09 ± 0.01
triglycerides	44.6 ± 1.2[ab]	49.4 ± 1.9[b]	40.9 ± 3.3[a]

[a] Values are means ± SEM; numbers in parentheses are the number of samples if they differ from the original sample size, n = 10. [b] Values with different superscript letters differ significantly ($p < 0.05$).

in that group. The average cecum weight of the animals fed M was 3.5 times higher than the weights observed in the control group. Liver weights did not differ between the CM and C group but were higher in the CM than in the M group. The average intestinal mucosa weight of the animals fed M was significantly higher than those of the control and CM groups. The weight of the total intestinal tract per 100 g of body weight was significantly correlated with the dietary MRP level (Figure 1).

Plasma, Lipoprotein, and Liver Analyses. The average cholesterol and triglyceride levels in the plasma and liver are shown in Table VI. Plasma cholesterol was highest in the group consuming the M diet. This value was significantly higher than those for the C and the CM groups. There were no significant differences in plasma triglyceride concentrations among the three groups. Animals fed the CM diet had significantly higher liver cholesterol than the control group. No significant difference was seen, however, between the M and C groups. The liver triglycerides were significantly higher in the CM than in the M group but did not differ from the C group.

Table VII. Composition of Lipoproteins (Milligrams per Deciliter of Plasma)[a,b]

	5% cellulose (C)	2.5% cellulose + 2.5% MRP (CM)	5% MRP (M)
HDL			
cholesterol			
total	14.8 ± 1.3	15.1 ± 1.3	15.8 ± 1.1
free	8.0 ± 1.1	8.3 ± 1.2	9.2 ± 1.5
esterified	6.87 ± 0.35	6.83 ± 0.54	6.91 ± 0.92
triglycerides	8.9 ± 1.9	6.7 ± 1.7	5.7 ± 0.9
proteins	41.2 ± 2.3	42.1 ± 2.4	42.2 ± 2.5
LDL			
cholesterol			
total	13.0 ± 2.5	10.9 ± 2.2	15.4 ± 1.4
free	2.2 ± 0.6	2.9 ± 1.1	3.4 ± 0.4
esterified	10.8 ± 1.9	8.1 ± 1.8	12.0 ± 1.0
triglycerides	2.6 ± 0.4	2.5 ± 0.3	2.9 ± 0.3
proteins	11.8 ± 1.2	10.9 ± 1.3	14.0 ± 0.8
VLDL			
cholesterol			
total	55.3 ± 4.5	51.4 ± 5.0	63.2 ± 3.4
free	13.1 ± 1.7[ab]	11.6 ± 1.2[a]	17.9 ± 3.1[b]
esterified	42.2 ± 3.2	39.8 ± 3.9	45.4 ± 2.4
triglycerides	35.1 ± 4.2	33.8 ± 3.7	33.7 ± 4.5
proteins	22.1 ± 1.5[ab]	20.2 ± 1.2[a]	24.9 ± 1.7[b]

[a] Values are means ± SEM; n = 10. [b] Values with different superscript letters differ significantly ($p < 0.05$).

There was no significant difference in the plasma levels of apo AI and apo B among groups, as can be seen in Table VI. Apo B concentrations were high compared to levels found in rats fed a diet without cholesterol or cholic acid. Apo AI levels, however, were in the normal range (Ney et al., 1988).

The plasma lipoprotein composition is presented in Table VII. Cholesterol was highest in the VLDL and relatively low in the HDL fraction. This effect was due to the nature of the model chosen in this experiment in which all groups were fed diets that contain cholesterol and cholic acid. No significant differences were observed among diet groups, even though total cholesterol concentrations of all lipoproteins followed the trend observed in the plasma and tended to be higher in the M group. Triglyceride concentrations were highest in the VLDL fraction but did not differ significantly among groups. The HDL fraction was the most concentrated in protein followed by the VLDL and the LDL fractions. Again, no significant difference was noted between the two experimental groups and the control.

Gradient gel electrophoresis was used to examine the size distribution in the HDL fraction. Our results showed that MRPs did not modify significantly the relative proportion of HDL_1 and HDL_2 or their diameters, compared to the control.

DISCUSSION

From a survey of the literature, it appeared that Maillard reaction products share some physical and physiological properties with dietary fibers. On the basis of this observation, we hypothesized that, like some sources of dietery fiber, MRPs could adsorb bile acids, interfere with their enterohepatic circulation, and lower plasma cholesterol. Two experiments were carried out to test these hypotheses. First, the in vitro adsorption capacity of several Maillard reaction systems was estimated. Second,

we conducted a feeding study to examine the effect of MRP on serum and hepatic cholesterol levels in rats. The Maillard reaction systems investigated in vitro were obtained by heating casein, egg albumen, or L-lysine with glucose. One system contained only the high molecular weight fraction of a glucose–L-lysine reaction mixture; this fraction was obtained by centrifugation with absolute ethanol.

The bile acid binding capacity of Maillard reaction systems is comparable with that of some sources of dietary fiber. In 100 mg of the glucose–L-lysine reaction mixture, L2, 13% of the cholic acid and 29% of the chenodeoxycholic acid were bound. Using a similar binding system, Story and Kritchevsky (1978) found that alfalfa and lignin adsorbed 20% and 44% cholic acid and 25% and 23% chenodeoxycholic acid, respectively. They concluded that alfalfa and lignin exhibited a considerable bile acid adsorption capacity. Hence, relative to the degree of bile acid binding exhibited by sources of fiber, MRPs display significant bile acid binding properties in vitro. By examining the bile acid binding capacities of different molecular weight fractions of MRP, we observed that the presence of compounds with molecular weights between 3000 and 30 000 did not notably enhance the degree of bile acid binding by MRP. Hence, MRPs with high molecular weights are responsible for the majority of the adsorption. Finally, the in vitro experiments demonstrated that MRPs, like many fiber sources (Eastwood and Hamilton, 1968; Story et al., 1982), have a greater affinity for chenodeoxycholic acid, a dihydroxy bile acid, than for cholic acid, a trihydroxy bile acid, suggesting that the adsorption is hydrophobic in nature. The results of the in vitro study suggest that bile acid adsorption by MRP, and more specifically high molecular weight MRP, could be of sufficient magnitude to alter sterol balance.

The MRPs that exhibited the highest level of bile acid adsorption in vitro were prepared in large quantities for incorporation into the diets to be fed in the in vivo experiment. These MRPs consisted of the compounds isolated from the glucose–L-lysine reaction mixture described as L2. Their molecular weight distribution was analyzed by gel chromatography. Absorbance at 300 nm was used to follow the elution profile of the material. In addition, the MRP was recovered to calculate the relative proportions (w/w) of the various molecular weight fractions. In agreement with Wu et al. (1987), a large portion of the MRPs have molecular weights above 10 000.

The objective of the in vivo experiment was to test whether MRP could exert a cholesterol-lowering action. Hence, rats were made hypercholesterolemic by adding 1% cholesterol and 0.2% cholic acid to the diets that were then supplemented with either 0, 2.5, or 5% MRP. The cholesterol and cholic acid model had been used previously in studies designed to test the ability of various sources of dietary fiber to prevent cholesterol accumulation (Chen et al., 1981; Jennings et al., 1988; Ney et al., 1988; Shinnick et al., 1988). Cholic acid increases cholesterol adsorption and inhibits its degradation. Therefore, feeding cholic acid in addition to cholesterol overcomes the resistance of rats to hypercholesterolemia.

As observed previously (Ney et al., 1988), the high cholesterol and cholic acid contents of the diets caused low cholesterol concentrations in the HDL fraction (15 mg/dL of plasma) and high cholesterol levels in the VLDL fraction (55 mg/dL of plasma). These values are comparable to the concentrations observed by Ney et al. (1988) and suggest that the fatty liver induced under such conditions may increase the production of VLDL particles to transfer the lipids accumulated into the circulation. This is in agreement with the high plasma concentrations of apo B, a major VLDL apolipoprotein, found in our study. In all groups, apo B concentrations in the plasma were found to be approximately 5–10 times higher than the usual range observed in rats fed a control diet without cholesterol or cholic acid (Ney et al., 1988). The elevated apo B concentrations support the hypothesis of the presence of more numerous VLDL particles in the plasma. The concentrations of apo AI, which is a major HDL apolipoprotein, were in the normal range (40–70 mg/dL). This value agrees with the results obtained by Ney et al. (1988). Apo AI may be synthesized normally even though the hypercholesterolemic diets decreased cholesterol levels in the HDL fraction, or apo AI can be associated with lipoprotein fractions other than HDL.

The presence of MRP in the diets failed to lower liver and plasma cholesterol levels in rats and even caused a modest accumulation of cholesterol. Other materials, such as wheat bran, bind bile acids in vivo (Story and Kritchevsky, 1976) but do not have a hypocholesterolemic effect in humans (Durrington et al., 1975). These findings could be due to the fact that the in vitro bile acid binding assay does not always accurately predict the extent of bile acid binding under physiological conditions. Additionally, bile acid binding is perhaps not the unique mechanism by which some fibers lower cholesterol. Fibers may also alter cholesterol adsorption by binding other micellar components in the small intestine (Gallaher and Schneeman, 1986; Ebihara and Schneeman, 1989). In addition, it is possible that fibers produce a change in the adsorptive capacity of the intestinal tract by interacting directly with the mucosa (Cassidy et al., 1981). Therefore, bile acid adsorption alone may not be sufficient to cause a cholesterol lowering effect, and more information is needed about the specific interaction of MRP with bile acids and micelles in the small intestine.

The lysine concentration of the Maillard reaction mixture incorporated in the diets was 43.7% (w/w). Hence, in our study, the lysine contents of the diets were 1.1% (CM diet) or 2.2% (M diet). Several studies have been carried out to elucidate the role of L-lysine on lipid metabolism (Hevia et al., 1980a,b; Jarowski and Pytelewski, 1975; Raja and Jarowski, 1975). According to Hevia et al. (1980a,b), at these levels, lysine supplementation does not influence serum and hepatic cholesterol or triglyceride levels. However, the total protein content of our diets differed from their study, and we included cholesterol and cholic acid in the diets to induce a hypercholesterolemic state. It seems unlikely that the lysine in the experimental diets influenced the results since the group with the highest dietary lysine had liver lipid levels comparable to those of the control group and in the CM group liver triglyceride and cholesterol concentrations were slightly higher than those of the control.

Consumption of 5% MRP resulted in higher weights of the intestinal tract and specifically of the small intestine and cecum plus contents. The significant correlation between intestinal weight and dietary MRP level suggests that, even at low levels, MRPs contribute to intestinal enlargement. The effect of MRP on the cecum size has been observed earlier (O'Brien and Walker, 1988; Pintauro et al., 1983) and could be due to an accumulation of undigested material or an increase of bacterial mass in the cecum. The small intestine and mucosa weights of the animals fed the M diet were higher than those of the animals fed the control diet. The mucosa of this experimental group had a darker color than that of the control

group, suggesting that some MRPs were present in this tissue. However, small concentrations of MRP can cause a very dark color and food had been withheld for 15 h, making it unlikely that the brown material associated with the mucosa was substantial enough to influence mucosa weights. Therefore, tissue hypertrophy or hyperplasia might have caused the high mucosa weights observed in the M group. Fiber supplementation is known to influence intestinal morphology. Brown et al. (1979) found that the length and weight of the small intestine were significantly greater in pectin-fed rats compared to that of a control group fed a fiber-free diet. The effect of pectin on the intestinal length has also been mentioned by Cassidy et al. (1986). Elhsenhans et al. (1981) found that guar, when compared to other gelling agents, produced the greatest increase in small intestinal mucosal mass. Jacobs (1983) observed that a diet containing 10% guar produced a significant increase in small intestinal mucosal mass. Fiber (mixed vegetables or from oats or cellulose) caused higher small intestine and colon weights compared to a control but did not affect cecum weights (Younoszai et al., 1978). Jacobs and Schneeman (1981) demonstrated that the feeding of a wheat bran diet produced colonic mucosal cell hyperplasia. Our results suggest that MRPs have effects on intestinal growth similar to those of some sources of dietary fiber. Nevertheless, more data on the influence of MRP on intestinal cell proliferation are needed to confirm this observation.

In conclusion, MRPs appear to have properties similar to those of a source of insoluble fiber, such as wheat bran, causing an enlargement of the cecum and binding bile acids but not lowering cholesterol levels in a hypercholesterolemic rodent model.

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Registry No. Cholesterol, 57-88-5; cholic acid, 81-25-4; chenodeoxycholic acid, 474-25-9.