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# Digestion of plant monogalactosyldiacylglycerol and digalactosyldiacylglycerol in rat alimentary canal

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*We investigated digestion of orally fed galactoglycerolipids such as monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) from wheat flour in the rat alimentary canal, especially focusing on the digestive fates of deacylated galactosylglycerol structures. After a single oral administration of MGDG (20 mg/rat), monogalactosylmonoacylglycerol and monogalactosylglycerol (MGG) were found to be major digestion products in the intestinal tract. Similarly, digalactosylmonoacylglycerol and digalactosylglycerol (DGG) were confirmed to be present in the intestinal tract after DGDG ingestion (20 mg/rat). In rats fed wheat flour glycolipids (42 mg MGDG and 81 mg DGDG per rat), completely deacylated galactosylglycerols (MGG and DGG) were not detected in portal plasma. Although the deacylated galactosylglycerols were not significantly decomposed by intestinal mucosa in vitro, they were hydrolyzed by cecal contents. The results demonstrated that orally ingested plant galactoglycerolipids in the rat alimentary canal are rapidly hydrolyzed into constituent fatty acids and that hydrophilic galactosylglycerols and the hydrophilic backbone galactosylglycerols are not absorbed from intestine or degraded into galactose and glycerol in the intestinal tract. Therefore, the presence of deacylated galactosylglycerols may affect the fermentative activity of enterobacteria in the cecum and colon.*


**Keywords**: digalactosyldiacylglycerol; digestion; intestine; monogalactosyldiacylglycerol; rats

## Introduction

Galactosylglycerolipids, particularly monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), are major lipid components in the thylakoid membranes of chloroplasts within plant leaves and are also present in grains, roots, and fruits.[1,2] These galactolipids exhibit galactosyl binding via β-1,3 linkage to glycerol. Because glycerolglycolipids usually are ingested daily from vegetable foods, it is thought that they may play a nutritional role in humans.

Several studies have investigated the digestion of glycerolglycolipids. An early study by Bajwa and Sastry[3] showed that acetone-dried powdered sheep pancreas preparations hydrolyzed MGDG and DGDG into free fatty acids and into the lyso compounds monogalactosylmonoacylglycerol (MGMG) and digalactosylmonoacylglycerol (DGMG), respectively, and further to monogalactosylglycerol (MGG) and digalactosylglycerol (DGG), respectively, and finally to galactose and glycerol. Human duodenal contents and pancreatic juice have been shown to hydrolyze MGDG, DGDG, and sulfoquinovosyldiacylglycerol, and free fatty acid liberation was confirmed.[4] Pancreatic lipase-related protein 2 has been shown to hydrolyze the ester linkage of galactoglycerolipids.[5] However, the hydrolytic cleavage of galactosylglycerol backbone structures occurs in the digestive tract and their metabolic fate has never been explained. A study using lymphatic duct-cannulated rats fed radiolabeled DGDG showed that no intact DGDG appeared in chyle lipids and that most of the radioactivity was found in the triacylglycerol fraction of lymph.[6] Despite one study that used fatty acid labeled DGDG, the metabolic fate of hydrophilic galactosylglycerol structures remains to be clarified.

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The goal of this study was to investigate the digestive fate of orally ingested wheat flour MGDG and DGDG in the rat alimentary canal, especially focusing on deacylated galactosylglycerol structures.

## Methods and materials

### *Preparation of wheat flour galactoglycerolipids*

The galactoglycerolipids MGDG and DGDG that were used for oral administration were extracted and refined from wheat flour. Wheat flour purchased from a local market was mixed with three volumes of acetone and let stand overnight. After filtration through filter paper (No. 2, Toyo Roshi Kaisha, Ltd., Tokyo, Japan), the acetone extract was concentrated by drying in a rotary evaporator. The concentrated acetone extract was applied to a silica gel column [500 mm × 30 mm of inner diameter (i.d.)] and chromatography was performed using chloroform (600 mL) and acetone (600 mL), successively, as eluents. The galactoglycerolipid fraction, recovered by acetone extraction, was further chromatographed in a silica gel column (500 mm × 30 mm i.d.) by stepwise elution with chloroform:acetone mixtures (9:1, 300 mL; 8:2, 200 mL; 7:3, 300 mL; 4:6, 200 mL; 2:8, 400 mL; 0:10, 400 mL).[7] MGDG was recovered in the 4:6 (v/v) chloroform:acetone extract and DGDG in the acetone extract. The purity of isolated galactolipids were confirmed using a Jasco high performance liquid chromatography (HPLC) system equipped with an evaporative light-scattering detector (ELSD; SEDEX model 55, Sedere, Virty sur Seine, France) under conditions described in the lipid analysis section. Single sharp peaks with the same retention times as those of either standard MGDG or DGDG (Sigma Chemical Co., St. Louis, MO USA) were verified for the isolated wheat flour galactolipids. Constituent fatty acids of wheat flour MGDG were 4.7% 16:0, 0.7% 18:0, 9.3% 18:1 (n-9), 81.3% 18:2 (n-6), and 4.0% 18:3 (n-3), and those of DGDG were 12.5% 16:0, 1.3% 18:0, 7.7% 18:1, 73.4% 18:2, and 5.1% 18:3.

The completely deacylated galactosylglycerols MGG and DGG were prepared by saponification[8] of approximately 200 mg each of wheat flour MGDG and DGDG dissolved in a mixture of 2 mL chloroform and 3 mL methanol. Saponification was carried out in 5 mL 0.2 M methanolic NaOH at room temperature for 20 minutes. After the reaction, 8 mL chloroform, 9 mL water, and 2 mL methanol were added, shaken, and centrifuged, and the upper aqueous phase recovered and neutralized with cation-exchange resin (Amberlite IRA-400, Organo Co., Tokyo, Japan). Using this method 46.7 mg of MGG and 60.7 mg of DGG were prepared.

### *Oral administration of galactoglycerolipids*

Six-week-old Male Sprague-Dawley rats were obtained from Funabashi Farm Co. (Chiba, Japan) and housed at 25°C with a 12-hour light-dark cycle with free access to commercial chow (F-2 pellet rations from Funabashi Farm Co.) and distilled water for 1 week. All animals were treated in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.[9] Rats were then weighed and allowed free access to water only overnight. Body weights were between 200 and 225 g.

In the first experiment, rats ($n = 24$) were fed 20 mg MGDG or DGDG dispersed in 2 mL water containing 0.5% sodium deoxycholate (Nacalai Tesque, Inc., Kyoto, Japan) by gastrogavage. After 1, 2, 3, or 6 hours, the rats were anesthetized with diethyl ether, and blood was collected from the abdominal artery with a heparinized syringe. Blood samples were centrifuged at 3,000 rpm for 15 minutes at 4°C to separate plasma. Aliquots of plasma (100 μL) were diluted with 2 mL methanol and centrifuged at 3,000 rpm for 20 minutes at 4°C to deproteinize. Plasma supernatants were concentrated in a rotary evaporator, redissolved in water, and filtered through a filter disc (pore size 0.45 μm, GL Sciences Inc., Tokyo, Japan). The filtered samples were applied to an ELSD-HPLC with a cation-exchange resin gel column (Shodex Ionpak KS-801, 300 mm × 8 mm i.d., Showa Denko, Tokyo, Japan) at 80°C with distilled water as column eluant at a flow rate of 0.7 mL/min to quantify glucose and galactose. Whole small intestines between the pylorus and cecum were removed and rinsed with cold saline. Intestinal contents were also collected by washing out with 40 mL cold saline.

In the second experiment, rats ($n = 3$) were fed 200 mg wheat flour glycolipid extracts containing 42 mg MGDG and 81 mg DGDG dispersed in 2 mL distilled water by gastrogavage. After 1 hour, portal blood (0.4 mL) was collected using an heparinized syringe, and plasma prepared by centrifugation at 3,000 rpm for 15 minutes at 4°C. Aliquots of the plasma (100 μL) were added to 2 mL methanol and centrifuged at 3,000 rpm for 20 minutes at 4°C to deproteinize. Plasma supernatants were concentrated in a rotary evaporator and redissolved in water. Plasma samples were applied successively to Sep-pak C18 cartridges, Accell Plus CM cartridges (cation exchange resin; Waters, Milford, MA USA), and Accell Plus QMA cartridges (anion exchange resin; Waters). All cartridges were washed out with 6 mL deionized/distilled water, and the final eluates were dried in a rotary evaporator. Dried residues were then redissolved in 100 μL distilled water and filtered through a cellulose nitrate filter disc (pore size 0.45 μm, GL Sciences Inc.). To analyze the completely deacylated galactosylglycerols MGG and DGG, the filtrates were applied to an ELSD-HPLC using an aminopropyl column (Shodex Asahipak NH2P50-4E, 250 mm × 4.6 mm i.d., Showa Denko) and acetonitrile:water (80:20, v/v) used as column eluant at a flow rate of 1.0 mL/min.

### *Analysis of galactoglycerolipids digestion with intestinal contents*

Lipids recovered using chloroform:methanol 2:1 (v/v) from 2 mL samples of intestinal contents collected from MGDG- or DGDG-fed rats were concentrated to dryness in a rotary evaporator and redissolved in chloroform. Intact galactolipids MGDG and DGDG and their corresponding lyso compounds MGMG and DGMG were quantified using a Jasco HPLC system combined with an ELSD detector (SEDEX model 55). A silica column (LiChrospher Si 60, 5 μm, 125 mm × 4 mm i.d., Merck, Darmstadt, Germany) was used with a binary gradient system using chloroform as the "A" solvent and methanol:water 95:5 (v/v) as the "B" solvent. Glycolipids were eluted using linear gradients of A:B from 99:1 to 75:25 over 15 minutes, to 10:90 in 5 minutes followed by a 10-minute hold. The flow rate was 1.0 mL/min. To quantify deacylated MGG and DGG, 10 mL methanol was added to 2 mL intestinal content samples and centrifuged at 3,000 rpm for 15 minutes at 4°C. Supernatants were concentrated in a rotary evaporator, and the dried residues were redissolved in water and filtered through a cellulose nitrate filter disc (pore size 0.45 μm) to eliminate impurities. The collected deacylation products MGG and DGG were applied to an ELSD-HPLC with an Ionpak KS-801 at 80°C with distilled water as column eluant at flow rate of 0.7 mL/min.

### *Digestion of deacylated galactosylglycerols with intestinal mucosa and cecal contents*

Small intestines and cecum were excised from 6-week-old male Sprague-Dawley rats (Funabashi Farm Co.) under ether anesthesia. The whole small intestines from pylorus to cecum were split open and rinsed with cold saline. The mucosa was scraped off with glass slides and homogenized with a Teflon-glass homogenizer in three volumes of cold saline. The cecal contents were washed out, collected in 20 mL cold saline, vortexed thoroughly, and centri-

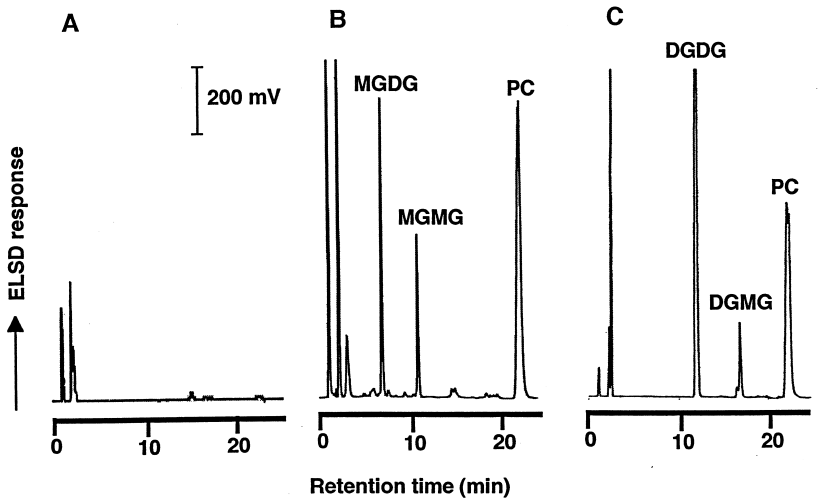


**Figure 1** Evaporative light-scattering detector high performance liquid chromatograms of lipid extract from intestinal contents of the galactosylglycerolipid-ingested rat. (A) The lipid extract from intestinal contents of the control rat. Lipid extract from intestinal contents 1 hour after administration of a single oral dose of (B) 20 mg monogalactosyldiacylglycerol (MGDG) and (C) 20 mg digalactosyldiacylglycerol (DGDG). Good reproducibility for the chromatogram was confirmed by each of the three rats. MGMG, monogalactosylmonoacylglycerol; DGMG, digalactosylmonoacylglycerol; PC, phosphatidylcholine.

fuged at 1,000 g for 20 minutes, and the supernatants were used for incubation. The mucosal homogenates and cecal content supernatants were assayed for protein by the method of Lowry et al.[10] with reagents obtained from Bio-Rad (Richmond, CA USA), and used as hydrolytic enzyme solutions for assay of hydrolytic cleavage of MGG, DGG, and lactose (control). The reaction mixture consisted of 0.1 mL enzyme solution and 28 mM substrate dispersed in a total of 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and incubated at 37°C for 1 hour. Time-course changes of hydrolytic products from 1 mM substrate by cecal contents also were measured after incubation at 37°C for 1, 3, 6, and 18 hours. Reactions were stopped by adding 3 mL methanol and centrifuged at 3,000 rpm for 20 minutes. Supernatants were dried in a rotary evaporator, and residues redissolved in 100 μL distilled water and passed through a filter disc. Galactose liberation was measured by ELSD-HPLC using an Ionpak KS-801 column as described above with the same analytical conditions as were used for the deacylated galactosylglycerols.

## Results

### *Intestinal deacylation of galactosylglycerolipids*

*Figure 1* shows ELSD-HPLC chromatograms for the lipid extracts from the rat small intestinal contents before and 1 hour after a single oral administration of MGDG and DGDG. MGDG was hydrolyzed and converted to MGMG as a lyso compound in the intestine. Similarly, DGDG also was hydrolyzed, yielding DGMG. A new peak component ascribed to endogenous phosphatidylcholine from bile was also found in the chromatograms after galactolipid administration.

*Figure 2* shows ELSD-HPLC chromatograms for the water-soluble compounds recovered from small intestinal contents before and 1 hour after a single oral administration of MGDG and DGDG. The formation of completely deacylated compounds such as MGG and DGG was confirmed. A galactose peak at 10.9 minutes of retention time was not observed. When the rats received DGDG orally, the partial hydrolysate of the galactosyl linkage, such as MGG, was not detected in water-soluble extracts of intestinal tract contents (*Figure 2C*).

Completely deacylated products MGG and DGG were predominant in the intestine 1 to 6 hours after galactolipid ingestion (*Figure 3*). Levels of intact galactolipids (MGDG, DGDG) and lyso compounds (MGMG, DGMG) were relatively small compared with galactosylglycerol levels. MGMG was not detected after 3 hours, but DGMG was found in the small intestine 6 hours after ingestion. Plasma galactose levels (26.4 ± 6.3 μg/mL) were unchanged even after oral supplementation of the plant galactoglycerolipids.

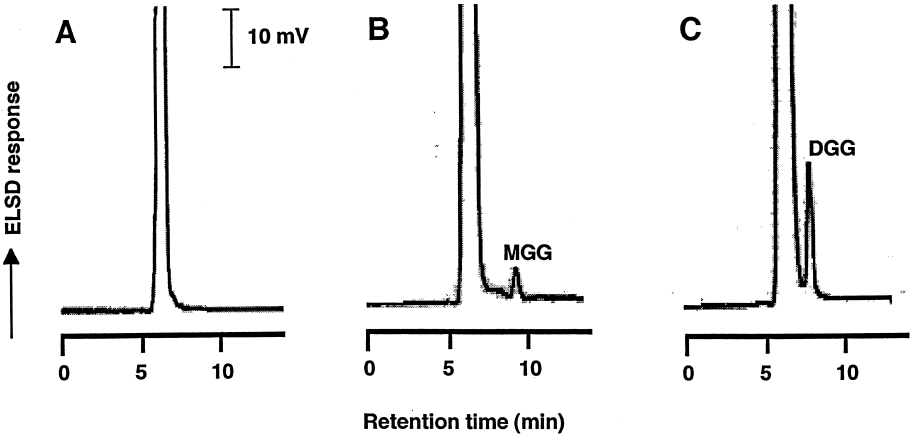


**Figure 2** Evaporative light-scattering detector high performance liquid chromatograms of water soluble extract from intestinal contents of (A) the control rat and rats 1 hour after a single oral administration of (B) 20 mg monogalactosyldiacylglycerol (MGDG) or (C) 20 mg digalactosyldiacylglycerol (DGDG). Good reproducibility for the chromatogram was confirmed by each of the three rats. MGG, monogalactosylglycerol; DGG, digalactosylglycerol.

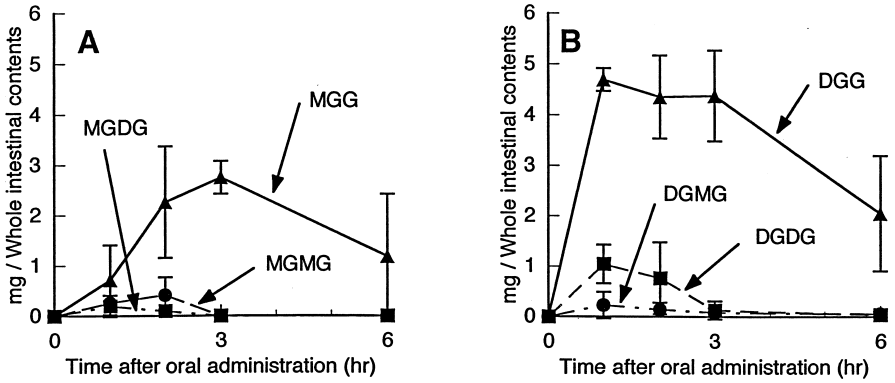


**Figure 3** Time course changes of degradation products in small intestine of the rat after a single oral administration of (A) 20 mg monogalactosyldiacylglycerol (MGDG) or (B) 20 mg digalactosyldiacylglycerol (DGDG). Values are mean ± SD of three rats at every each point. MGMG, monogalactosylmonoacylglycerol; DGMG, digalactosylmonoacylglycerol; PC, phosphatidylcholine; MGG, monogalactosylglycerol; DGG, digalactosylglycerol.

### *No intestinal absorption of completely deacylated galactoglycerolipid products*

To estimate intestinal absorption of MGG and DGG, portal plasma samples were collected and analyzed by ELSD-HPLC. Rats were orally administered wheat flour glycolipids (42 mg MGDG, 81 mg DGDG/rat), so that the calculated amounts of MGG and DGG that originated from the orally fed glycolipids were 14 mg and 36 mg, respectively. As shown in *Figure 4*, the completely deacylated products MGG and DGG were not detected in portal plasma after galactoglycerolipid administration (<5 μg/mL MGG and <10 μg/mL DGG).

### *Hydrolytic cleavage of galactosylglycerol galactosyl linkages by cecal contents*

Hydrolytic activity associated with small intestinal mucosa and cecal contents on the galactosyl linkage in MGG and DGG was evaluated by measuring liberated galactose by ELSD-HPLC. The liberation of galactose (μmol/mg protein/hr) from MGG and DGG was compared with galactose release from lactose. The results revealed that, although MGG and DGG were not hydrolyzed by the small intestinal mucosa, cecal contents hydrolyzed both galactosylglycerols into galactose and glycerol at approximately one- to two-thirds of the hydrolytic activity for lactose (*Table 1*).

MGG was completely hydrolyzed by the cecal contents within 6 hours to galactose and glycerol (*Figure 5*), whereas the hydrolytic degradation of DGG occurred at a slower rate. On exposure to cecal contents, MGG was also formed from DGG. After 18 hours of incubation, detectable levels of MGG, DGG, and galactose had completely disappeared.

## Discussion

As shown in the present study, the ELSD-HPLC method is a very useful technique for investigating the digestive fates of MGDG and DGDG. Orally fed plant galactosylglycerolipids MGDG and DGDG were rapidly hydrolyzed to their corresponding deacylation products (MGG and DGG) in the rat intestinal tract. MGG and DGG were indigestible in the intestine and very little was absorbed in portal blood via the intestinal tract. However, it was shown that in the cecum both MGG and DGG were further digested into galactose and glycerol.

Previous studies have investigated the digestion and intestinal absorption of sugar-containing lipids such as cerebroside,[11] sterylglucoside,[12] authentic alkyl β-glycoside,[13] and glyceroglycolipids.[3–6] In an early study, Bajwa and Sastry[3] investigated the degradation pathway of MGDG and DGDG using pancreas homogenates in vitro. According to their findings, the major pathway of galactoglycerolipid

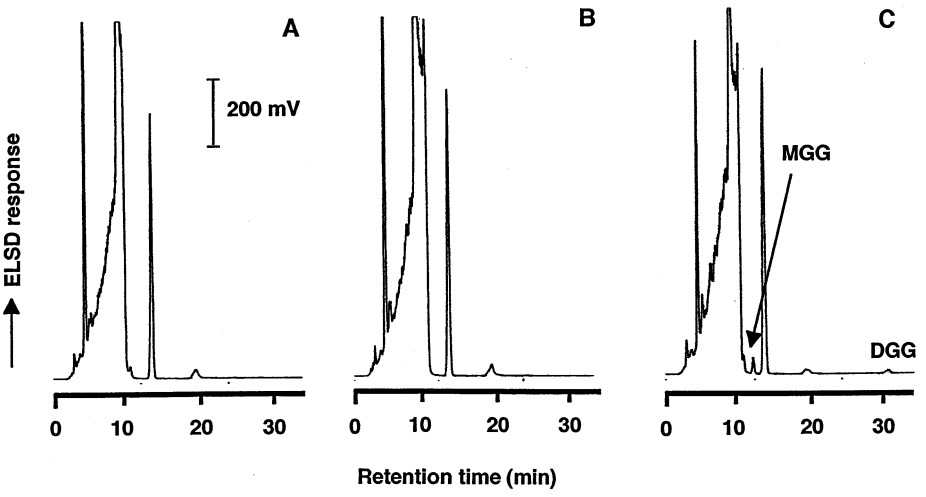


**Figure 4** Evaporative light-scattering detector (ELSD) high performance liquid chromatography (HPLC) of water soluble extract from portal blood plasma (A) of the control rat and (B) of rat 1 hour after a single oral administration of 200 mg wheat flour glycolipid extract. Good reproducibility for the chromatogram was confirmed by each of the three rat. On the other hand, each 0.5 μg of monogalactosylglycerol (MGG) or digalactosylglycerol (DGG) was exogenously added to portal blood plasma of control rat and the water soluble extract was submitted to ELSD-HPLC (C).

**Table 1** Digestive hydrolysis of deacylation products of galactoglycerolipids with rat cecal contents and intestinal mucosa

| Substrate | Cecal contents (Gal μmol/mg protein/hr) | Intestinal mucosa (Gal μmol/mg protein/hr) |
|---|---|---|
| Lactose | 4.31 ± 1.26 | 0.47 ± 0.09 |
| MGG | 2.67 ± 0.38 | <0.01 |
| DGG | 1.70 ± 0.83 | <0.01 |

28 mM of each substrate was incubated with cecal contents (ca. 0.4 mg protein/mL) or intestinal mucosa (ca. 6 mg protein/mL) in 10 mM potassium phosphate buffer (pH 7.0) at 37°C for 1 hour.
Values are means ± SD of three rats.
MGG–monogalactosylglycerol. DGG–digalactosylglycerol.

degradation was postulated to be the hydrolysis of the ester bonds to yield free fatty acids and intermediate partial deacylation products MGMG and DGMG. They also referred to degradation to MGG and DGG from the corresponding lyso compounds, and the final formation of galactose.[3] Andersson et al.[4] reported galactolipase activity in human duodenal contents and in pancreatic juice in vitro. They confirmed that DGDG was hydrolyzed to DGMG during incubation with human duodenal contents and that the lyso compound was not hydrolyzed further after a 2-hour incubation period. In contrast, our present in vivo study confirmed that both MGDG and DGDG were rapidly hydrolyzed to completely the deacylated products MGG and DGG in the rat intestinal tract. The partial deacylation products MGMG and DGMG were relatively minor products, and galactose liberated from the deacylation products was not found in the rat intestinal tract.

Recently, Ohlsson et al.[6] reported that orally fed DGDG was not intestinally absorbed in its intact form by rats. They also found that although fatty acids released from DGDG were incorporated into lymphatic triacylglycerols, the recovery from chyle lipids was low. Thus, the fatty acids were thought to be absorbed via the portal route rather than the lymph. However, the digestion and absorption of the DGDG deacylation products were not discussed. Earlier studies indicated that dietary glycerophospholipids are hydrolyzed to lyso compounds by pancreatic phospholipase $A_2$ in the intestinal lumen, and most of these lysoglycerophospholipids, such as lysophosphatidylcholine, are incorporated into intestinal mucosal cells and reacylated to form chylomicron glycerophosholipid.[14,15] However, in contrast to glycerophospholipids, DGDG was not absorbed from the intestine as reacylated monoacyl compound in the rat. [6] Ikeda et al.[16] reported that orally fed glycerophospholipids were rapidly hydrolyzed into their corresponding lysophospholipids, glycerophosphoryl bases, and constituent bases in the rat small intestine, and suggested that the latter two water-soluble compounds were absorbed via the portal vein. However, as shown in the present study, MGG and DGG, the deacylation products of MGDG and DGDG, respectively, remained in the intestinal tract for as long as 6 hours after intake (*Figures 2 and 3*). Furthermore, these galactosylglycerols were not detected in their intact forms in the portal plasma of rats administered wheat flour glycolipids. When galactose (4 g/kg) was administered orally to rats, portal plasma concentrations of galactose were reported to increase to 19.3 mM (3.5 mg/mL) after 60 minutes.[17] Although it may depend on the dose, the present study revealed that portal plasma concentrations of MGG and DGG were relatively low (<5 μg/mL MGG and <10 μg/mL DGG) compared with galactose absorption. Taking into consideration the time lag in intestinal deacylation, these findings nevertheless suggested that both MGG and DGG were indigestible structures in the intestinal tract in vivo. We also confirmed that MGG and DGG were barely hydrolyzed by intestinal mucosa in contrast with lactose (*Table 1*). The structural backbone of MGG is β-D-galactopyranosyl-1-glycerol and that of DGG is α-D-galactopyranosyl-(1→6)-β-D-galactopyranosyl-1-glycerol.[1] Thus, intestinal β-galactosidase (lactase) might not be able to efficiently hydrolyze the β-galactosyl linkages of MGG and DGG, leading to β-galactosyloligosaccharides being poorly digested in the small intestine.[18–20] We also confirmed that the α-galactosidic linkage of DGG was not hydrolyzed by the intestinal mucosa. It has been postulated that α-galactosidase does not exist in the mammalian small intestine.[21] However, the cecal contents were shown to have hydrolytic activity on MGG and DGG (*Table 1* and *Figure 5*), indicating the occurrence of α-galactosidase activity in cecal contents. This was further confirmed by evidence of galactose liberation from DGG. When MGG and DGG were incubated with the cecal contents for 18 hours, intact galactosylglycerols and their hydrolyzed products disap-

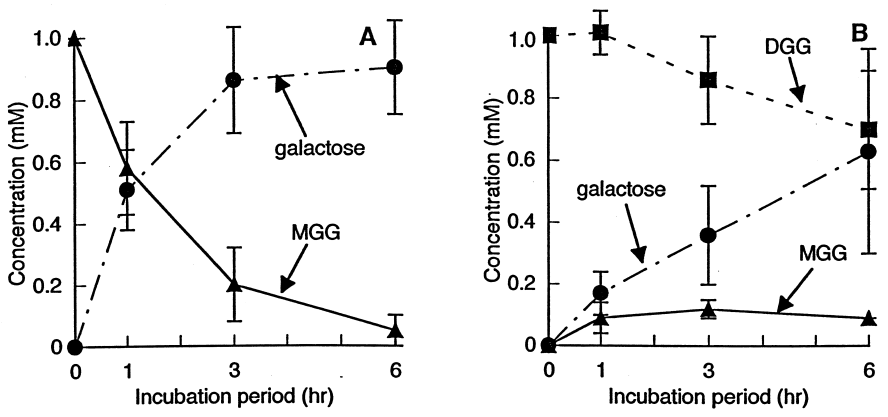


**Figure 5** Further degradation of deacylated products of galactoglycerolipids with rat cecal contents. Each 1 mM (A) monogalactosylglycerol (MGG) or (B) digalactosylglycerol (DGG) was incubated with rat cecal contents (ca. 0.4 mg protein/mL) at 37°C. Values are means ± SD of three rats at each time.

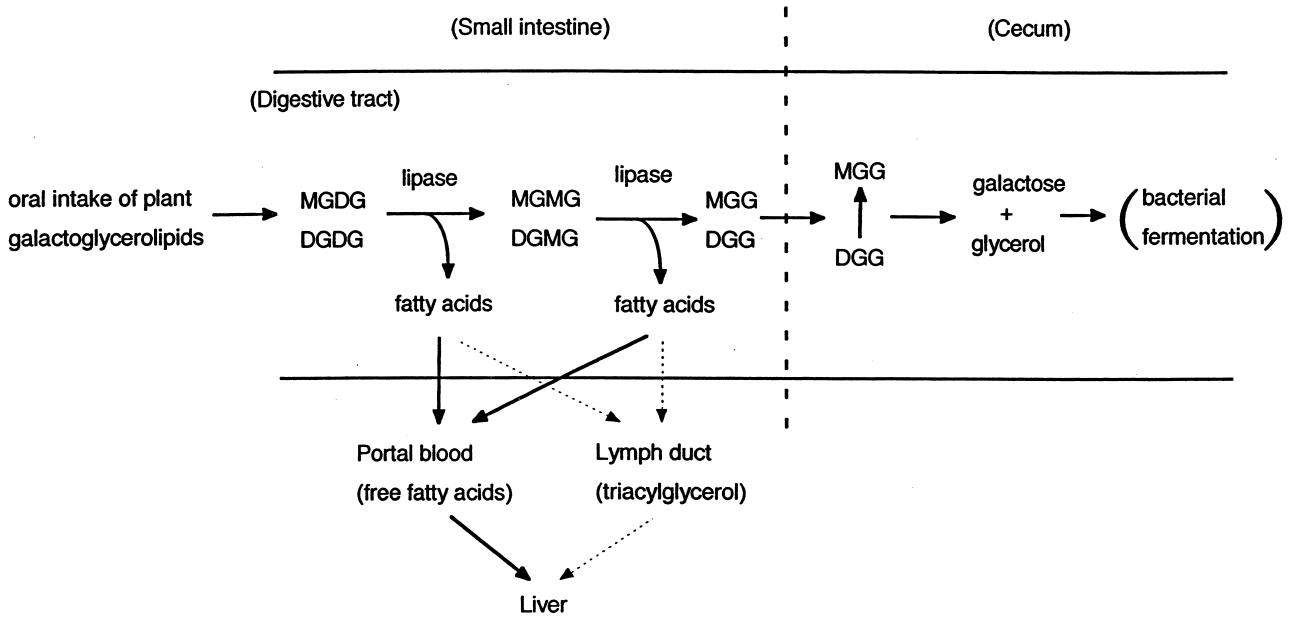


**Figure 6.** Digestion of plant galactoglycerolipids, MGDG, and DGDG, in rat alimentary canal.

peared, presumably due to fermentative enterobacteria. Thus, the beneficial effects of galactosylglycerols as deacylation products from dietary plant galactoglycerolipids would be expected to occur in the cecum and colon.

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