Hydrolysis of galactolipids by human pancreatic lipolytic enzymes and duodenal contents

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Abstract Monogalactosyldiacylglycerols (MGDG), digalactosyldiacylglycerols (DGDG) and sulfoquinovosyldiacylglycerols (SQDG) are major lipids in vegetable food. Their digestion and absorption are unknown. This study examines the hydrolysis of galactolipids in vitro with human duodenal contents, pancreatic juice, and purified human pancreatic lipases. Galactolipids were incubated with human duodenal contents, pancreatic juice, pure pancreatic carboxyl ester lipase (CEL), and colipase-dependent lipase with colipase (Lip-Col). Hydrolysis was estimated as release of free fatty acids and by the use of [^{3}H]galactose or [^{3}H]fatty acid-labeled DGDG. Pancreatic juice and duodenal contents hydrolyzed DGDG to fatty acids, digalactosylmonoacylglycerol (DGMG) and water-soluble galactose-containing compounds. The hydrolysis of DGDG was bile salt-dependent and had a pH optimum at 6.5–7.5. Human pancreatic juice released fatty acids from MGDG, DGDG, and SQDG. Purified CEL hydrolyzed all three substrates; the hydrolysis rate was MGDG > SQDG > DGDG. Pure Lip-Col had activity toward MGDG but had little activity against DGDG. Separation of pancreatic juice by Sephadex G100 gel filtration chromatography revealed two peaks with galactolipase activity that coincided with CEL (molecular mass 100 kD) and lipase (molecular mass 50 kD) peaks. In contrast to pure Lip-Col enzymes of the latter peak were as active against DGDG as against MGDG. Thus, DGDG is hydrolyzed both by CEL and by a pancreatic enzyme(s) with a molecular mass of 40–50 kD to fatty acids and lyso DGDG. MGDG, DGDG, and SQDG are all hydrolyzed by human pancreatic juice. Pure CEL hydrolyzed all three substrates.—**Andersson, L., C. Bratt, K. C. Arnoldsson, B. Herslöf, N. U. Olsson, B. Sternby, and Å. Nilsson.** Hydrolysis of galactolipids by human pancreatic lipolytic enzymes and duodenal contents. *J. Lipid Res.* 1995. **36:** 1392–1400.

Supplementary key words digalactosyldiacylglycerol (DGDG) • monogalactosyldiacylglycerol (MGDG) • sulphoquinovosyldiacylglycerol (SQDG) • digestion • carboxyl ester lipase (CEL) • lipase and colipase (Lip-Col)

Chloroplasts from higher plants contain thylakoid membranes where photosynthesis takes place. The thylakoid membranes are composed of 60–65% protein, and 35–40% lipid, depending on growth conditions. The lipid composition of the thylakoids is unique among eukaryotic cellular membranes because about 77% of the lipids are neutral galactosyldiacylglycerols (1). Of the total nonpigmented membrane lipids, about 51% are monogalactosyldiacylglycerols (MGDG) and about 26% are digalactosyldiacylglycerols (DGDG). The thylakoid membranes also contain sulfoquinovosyldiacylglycerol (sulfonated MGDG, SQDG). Galactolipids are also present in nonphotosynthetic tissues such as potato tuber, apples, and seed grains; in potato tubers 14.2% of the lipids are DGDG and 5.7% MGDG (2). The amount of phospholipids in nonphotosynthetic tissues consistently exceeds the amount of galactolipids (2, 3). Small amounts of other galactolipids have also been found in plant material such as trigalactosyldiacylglycerol and tetragalactosyldiacylglycerols (4, 5). Accordingly, galactolipids are important food constituents in both animals and humans and would be expected to be an important source of essential fatty acids, particularly in ruminants and other herbivores. For example, linolenic acid (18:3 n–3) accounts for more than 90% of the total fatty acids of galactolipids in most plant leaves (6), whereas galactolipids of other sources such as wheat and oat grains are rich in linoleic acid (18:2 n–6).

There is a lack of information concerning digestion and absorption of galactolipids. In this study we examined the digestion of galactosylglycerides, particularly DGDG, with human duodenal contents, pancreatic juice, and pure human pancreatic lipolytic enzymes.

Abbreviations: DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; SQDG, sulfoquinovosyldiacylglycerol; DGMG, digalactosylmonoacylglycerol; FFA, free fatty acids; TLC, thin-layer chromatography; CEL, carboxyl ester lipase; Lip-Col, colipase-dependent lipase and colipase.

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MATERIAL AND METHODS

Sources of galactolipids

A mixture of galactolipids from oat grains was obtained from Scotia LipidTeknik, Stockholm, Sweden. The composition was 75% DGDG and 25% other glycolipids including MGDG and SQDG. Chromatographically pure MGDG, DGDG, and SQDG, prepared from plant leaves, were obtained from Lipid Products, S. Nutfield Surrey, England. [^{3}H]UDP-galactose (NET-758, 30–50 Ci/mmol) was obtained from New England Nuclear. Silica gel 60 TLC plates, 0.5 mm thick, were obtained from Merck.

Preparation of [^{3}H]galactose-labeled DGDG

The method was based on studies of the galactolipid-synthesizing enzymes in pea seedlings by Siebertz and Heinz (7). Ten grams of frozen seedlings (1–1.5 cm) from *Capella* peas were crushed in a mortar in the presence of liquid nitrogen. The seedling powder was homogenized in cold acetone containing 3% Triton X100. The green supernatant was removed by filtration through a 0.2 μm nylon filter. Acetone extraction was then performed and continued until the green color disappeared. The residue acetone powder was then dried in a vacuum desiccator, weighed, and homogenized in 1 ml 0.1 M 2-(4-morpholino)ethansulphonate (MES) buffer, pH 6, containing 30% glycerol and 3% Triton X100 (25 mg acetone powder/ml). Two mg pure MGDG and 1.6 ml of the enzyme solution were mixed and sonicated for 4 × 20 sec with the tube kept on ice. After addition of 5 μCi (50 μl) [^{3}H]UDP-galactose the mixture was incubated at 32°C for 60 min. The incubation was interrupted by addition of 2 ml chloroform–methanol 2:1 to extract lipids, followed by 0.5 ml 0.45% NaCl. After centrifugation at 2000 rpm for 10 min, the upper phase was removed and the lower phase was washed once with methanol–water–chloroform 480:470:30. The lower phase was taken to dryness and redissolved in 2 ml chloroform containing 0.005% butylated hydroxytoluene. Analysis by thin-layer chromatography (TLC) on silica gel 60 plates, developed in chloroform–methanol–acetic acid–water 60:40:3:0.3 (8), showed that 96% of the lipid ^{3}H was in DGDG. The yield was 15–28% of the added [^{3}H]UDP-galactose.

Preparation of [^{3}H]fatty acid-labeled DGDG

Five hundred mg of the galactolipid mixture was labeled by catalytic reduction of double bonds in fatty acids with tritium gas. The labeling was performed by Amersham Tritium Labelling Service, England; the specific radioactivity was 30–60 Ci/mmol per double bond reduced. [^{3}H]DGDG was purified by two-dimensional TLC on silica gel 60 plates according to Bratt and Åkerlund (9). The mobile phases were chloroform–methanol–water 65:25:4 (v/v) in the first direction and chloroform–methanol–acetic acid–water 85:15:10:3.5 in the second direction. The DGDG spot was eluted sequentially with chloroform–methanol–water 65:25:4 and 50:50:10, pure methanol, and methanol–water 1:1. To determine the proportion of ^{3}H in fatty acids 18 nCi of ^{3}H-labeled DGDG and 2 mg unlabeled galactolipids were subjected to alkaline hydrolysis in 1 ml 1 M KOH at 60°C for 4 h. After neutralization with 200 μl 5 M HCl, 5 ml chloroform, 1.5 ml ethanol, and 2.5 ml water were added. In this two phase distribution 97% of the ^{3}H was in the lower (chloroform) phase. Of the lipid-soluble radioactivity, 89% migrated as free fatty acids while two other spots migrated equivalent to diacylglycerol (DG; 4%) and monoacylglycerol (MG; 7%), respectively, on silica gel G plates developed in light petroleum ether–diethylether–acetic acid 80:20:1. The hydrolysis products migrating as MG and DG are expected to be mono- and dihydroxyacids, respectively, which are present in the intact galactolipids as so-called estolides (10), i.e., galactolipids containing hydroxy acyl groups to which additional fatty acids are esterified.

Sources of enzymes and bile salts

Sodium-taurocholate (NaTC), sodium-taurodeoxycholate (NaTDC), sodium-glycocholate (NaGC), and sodium-glycochenodeoxycholate (NaGCDC) were synthesized by the method of Norman (11) (by Dr. Lennart Krabisch, Dept. of Physiological Chemistry, University of Lund). Human duodenal content was collected by duodenal intubation after ingestion of a liquid test meal (12). Its CEL activity was 68.3 μmol · ml^{-1} · min^{-1}, and the lipase and colipase activities were 923 and 838 μmol · ml^{-1} · min^{-1}, respectively, when assayed as described by Erlanson (13) and Sternby et al. (14). Human pancreatic juice was a gift from Docent Anders Borgström, Department of Surgery, Malmö General Hospital. The CEL activity was 4.2 μmol · min^{-1} · ml^{-1} and the lipase and colipase activities were 1025 and 562 μmol · ml^{-1} · min^{-1}, respectively. Human pancreatic carboxylesterlipase (CEL) was purified to homogeneity from human pancreatic juice according to the method of Lombardo, Guy, and Figarella (15). Lipase and colipase (lipase-colipase 1:1, w/w) were purified from human pancreas according to Sternby (16) and Sternby and Borgström (17). Polyclonal antiserum against CEL was prepared according to the method by Aho et al. (18). Pure phospholipase A_2 was purified from human pancreas according to Verheij et al. (19). Crude lipase from porcine pancreas, type II, was obtained from Sigma (# L3126). CEL and colipase-dependent lipase activities were determined according to Erlanson (13) and Sternby et al. (14).

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Separation of human pancreatic juice by gel filtration

Human pancreatic juice (2.4 ml) was separated on a K16/70 column packed with Sephadex G100 according to the method described by Lombardo et al. (15). Fractions of 1.6 ml were collected at a flow rate of 0.4 ml/min and protein concentrations were determined by micro BCA Protein Assay Reagent Kit (Pierce from Tectator).

Incubations of galactolipids with duodenal contents, pancreatic juice, and pure enzymes

All incubations were performed in 10 mM Tris-maleate buffer, pH 7, containing 2 mM $CaCl_2$ and 0.12 M NaCl, except in the experiments in which the effect of pH was studied. In these experiments we used acetate buffer at pH 5–6, Tris-maleate buffer at pH 6.5–7.5 and Tris-HCl buffer at pH 8–9. The bile salt concentrations are indicated in legends to tables and figures. In some initial experiments with duodenal contents, 1.5 mM NaTC and 2.0 mM NaTDC were used; in all other experiments various concentrations (3.2–9 mM) of a bile salt mixture containing 42% (w/w) NaTC, 26% NaTCDC, 22% NaGC, and 10% GCDC were used. Two mg galactolipid was dispersed in 2 ml incubation buffer with bile salts by sonicating for 4 × 30 sec using a Branson Sonifier 250 (KEBO lab, Sweden). The dispersion was preheated at 37°C and the reaction was started by adding aliquots of duodenal contents pancreatic juice, or pure enzymes. At the time intervals indicated, aliquots of the incubation mixtures were taken for analysis of FFA, analysis of fatty acid composition of substrates and products, or analysis of the distribution of radioactivity between substrate and reaction products as described below.

In one series of experiments the effect of a polyclonal antiserum towards human CEL was examined during hydrolysis of galactolipids by human pancreatic juice. After 4 min, 10 μl of the antiserum (18) or 10 μl control rabbit serum was added. The incubations were terminated by placing the samples on dry ice or in a −70°C freezer. The hydrolysis rate was determined by measuring released free fatty acids as described below.

Determination of galactolipid hydrolysis

Release of free fatty acids. Hydrolysis was measured by estimating the amount of free fatty acids released during the incubations, using an NEFA-C kit (WAKO Chemicals GmbH, Germany), which is based on an in vitro enzymatic method for the quantitative determination of nonesterified fatty acids. To avoid further hydrolysis during the NEFA-C kit reactions, the aliquots of the incubation mixture were heated to 100°C for about 5 min to inactivate lipases before analyzing released free fatty acids. This inactivation step was not carried out in the experimental series with pure lipases, presented in Fig. 6 and 7. At the enzyme concentrations used the inactivation made little or no difference. At the concentration of pancreatic juice used in the experiment shown in Fig. 5, increased zero-time values were seen without heat inactivation.

Hydrolysis of radioactive DGDG. When [^{3}H]galactose-labeled or [^{3}H]fatty acid-labeled DGDG was used as substrate the reaction was interrupted by extracting aliquots of the incubation mixture with chloroform–methanol 1:1 (20) containing 0.005% butylated hydroxytoluene at the time intervals indicated. Aliquots of the lipid extracts were separated on TLC plates developed in chloroform–methanol–acetic acid–water 60:40:3.0:0.3. Radioactivity of DGDG, DGMG (digalactosylmonoacylglycerol), and nonpolar lipids was determined as described earlier (8). Analysis of the upper phases after two-phase distribution in chloroform–methanol–water 2:1:1 indicated that 8% of the DGMG but negligible amounts of DGDG were partitioned to the upper phase. Accordingly, values for DGMG of the lipid phase were corrected by dividing by 0.92.

Characterization of the polar hydrolysis product of DGDG. From an incubation of galactolipids with diluted duodenal contents, lipids were extracted and separated by TLC. The spots of DGDG and DGMG were eluted sequentially as described above for the purification of [^{3}H]fatty acid-labeled DGDG. The eluates were taken to dryness with nitrogen. Half of each sample was subjected to acid hydrolysis in 1 ml 3 M HCl for 5 h at 90°C. After neutralization with 300 μl 10 M NaOH, free galactose was determined using a galactose UV-method Test combination (Boehringer Mannheim, Germany). The other half of each sample was subjected to alkaline hydrolysis in 1 M KOH in 95% ethanol for 4 h at 60°C. After neutralization with 200 μl 5 M HCl, unesterified fatty acids were determined by the NEFA-C kit.

HPLC analysis of galactolipids

In an initial experimental series, a galactolipid fraction (103 mg) from Karlshamns LipidTeknik AB, Stockholm, originating from dehulled oat seed (*Avena sativa* L.) was suspended in 10 ml KH_2PO_4/NaOH buffer at pH 7.0. Four hundred mg (18,400 U) of lipase powder (from porcine pancreas, Sigma Chemical Company (EC 3.1.1.3.)) was added and the mixture was stirred for 22 h at room temperature; thereafter 2 drops HCl (25%) was added. Free fatty acids were extracted with 2 × 3 ml isooctane. The galactolipid material remaining in the aqueous phase was analyzed by HPLC with light scattering detection as previously described before (21, 22).

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RESULTS

Hydrolysis of galactolipids with human duodenal contents

In initial experiments designed to find a method for identifying and characterizing the more polar products formed by the hydrolysis of galactolipids with triacylglycerol lipases, a major product tentatively identified as DGMG was demonstrated after long incubations with crude porcine pancreatic lipase (**Fig. 1**). During incubation of galactolipids containing [^{3}H]fatty acid-labeled DGDG with human duodenal contents for 2 h, the radioactivity of the DGDG spot decreased by more than 80% (**Fig. 2**). A new spot appeared migrating with an R_f value about 60% of that of DGDG. This spot contained significant radioactivity, which increased with time (Fig. 2A). Data from alkaline and acidic hydrolysis of this product showed an FFA/galactose ratio of 0.48 μmol/μmol indicating that it consisted of DGMG. The nonpolar radioactivity, mainly FFA, also increased with time. Some radioactivity migrated as mono- (MG) and diacylglycerols (DG). As this was also the case after alkaline hydrolysis of the [^{3}H]DGDG under conditions that would be expected to hydrolyze all ester bonds of galactolipids, we believe that this radioactivity represents release of ^{3}H-labeled hydroxy fatty acids (see Material and Methods). Data are therefore presented as radioactivity in total nonpolar lipids. In experiments in which [^{3}H]galactose-labeled DGDG had been added to the galactolipid mixture, the radioactivity in DGDG decreased with time and the radioactivity of the DGMG spot and water-soluble ^{3}H increased (Fig. 2B). The data thus indicated that enzymes of human duodenal contents hydrolyzed DGDG to DGMG and nonpolar lipids, mainly FFA. In addition, there was some formation of water-soluble galactose-containing compounds.

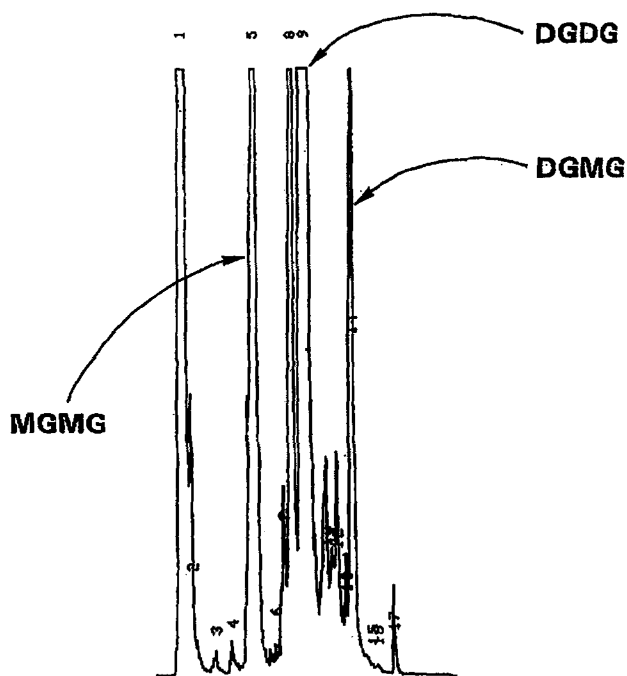
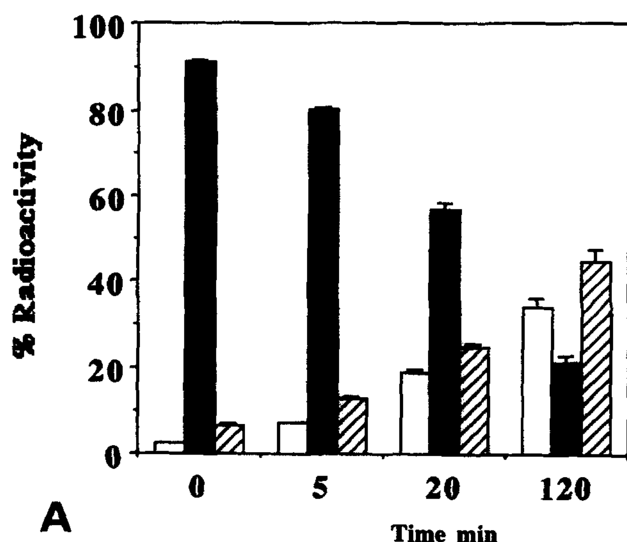


Fig. 1. HPLC chromatogram of a galactolipid fraction from oat seed after hydrolysis with porcine pancreas lipase. Incubation conditions are given in the Material and Methods section.



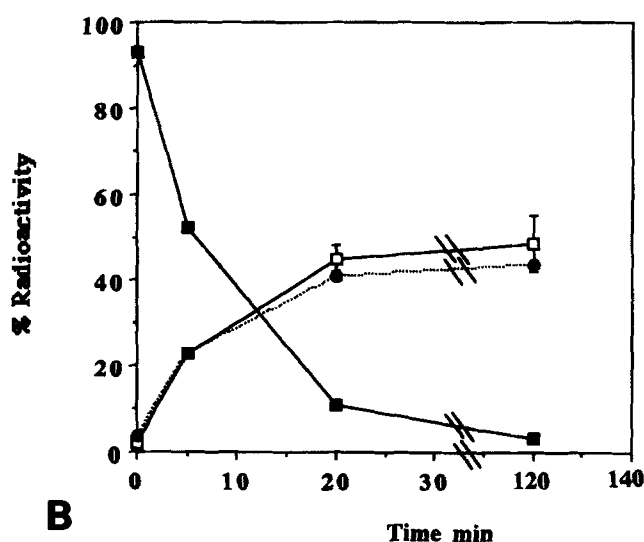


Fig. 2. A: Hydrolysis of [^{3}H]fatty acid-labeled DGDG with duodenal contents. Two mg galactolipids containing 44,000 dpm fatty acid-labeled DGDG was incubated with duodenal contents (0.63%) under the conditions given in the Methods section. The bile salt concentration was 6.4 mM. The results show % radioactivity in DGMG (□), DGDG (■), and nonpolar lipids (□) and are means ± SEM of three observations. B: Hydrolysis of [^{3}H]galactose-labeled DGDG with human duodenal contents. Two mg galactolipids containing about 27,000 dpm [^{3}H]galactose-labeled DGDG was incubated with duodenal contents (2.5%) under the conditions given in the Methods section. The bile salt concentration was 6.4 mM. The result show % radioactivity in DGDG (■), DGMG (□), and water-soluble compounds (●) and are means ± SEM of three observations.

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Hydrolysis of galactolipids with human pancreatic juice

These experiments were undertaken to determine whether the enzyme(s) in the duodenal contents that hydrolyzed DGDG were of pancreatic origin. The pancreatic juice efficiently hydrolyzed [^{3}H]fatty acid-labeled DGDG to free fatty acids and DGMG (**Fig. 3A**). The hydrolysis had a broad pH optimum between pH 6.5 and 7.5 (Fig. 3B). Without bile salts or with a bile salt concentration below 1.6 mM no measurable hydrolysis occurred. Bile salts (3.2–12.8 mM) stimulated the hydrolysis of DGDG, the highest rate being observed at 4.8 mM (Fig. 3C). Thus, there were enzymes dependent on bile salts for their action present in human pancreatic juice that hydrolyzed galactolipids. In one experimental series, galactolipids were incubated with pancreatic juice with either a blocking antiserum towards CEL or a corresponding control serum. In this experiment the anti-CEL caused a partial inhibition of the hydrolysis (**Fig. 4**), suggesting that CEL accounted for some of the galactolipid hydrolysis, although conclusions concerning the quantitative role of the enzyme could not be drawn because the antiserum did not block the hydrolysis of *p*-nitrophenylacetate. When the pancreatic juice was incubated with pure DGDG, MGDG, and SQDG, hydrolysis of all three substrates was observed. The rate was higher with DGDG and MGDG than with SQDG (**Fig. 5**).

Hydrolysis of galactolipids with different pancreatic enzymes

Pure CEL hydrolyzed mixed galactolipids in a concentration-dependent fashion (**Fig. 6**). The hydrolysis with pure human colipase-dependent lipase was slower (Fig. 6) and addition of Lip-Col to incubations with CEL

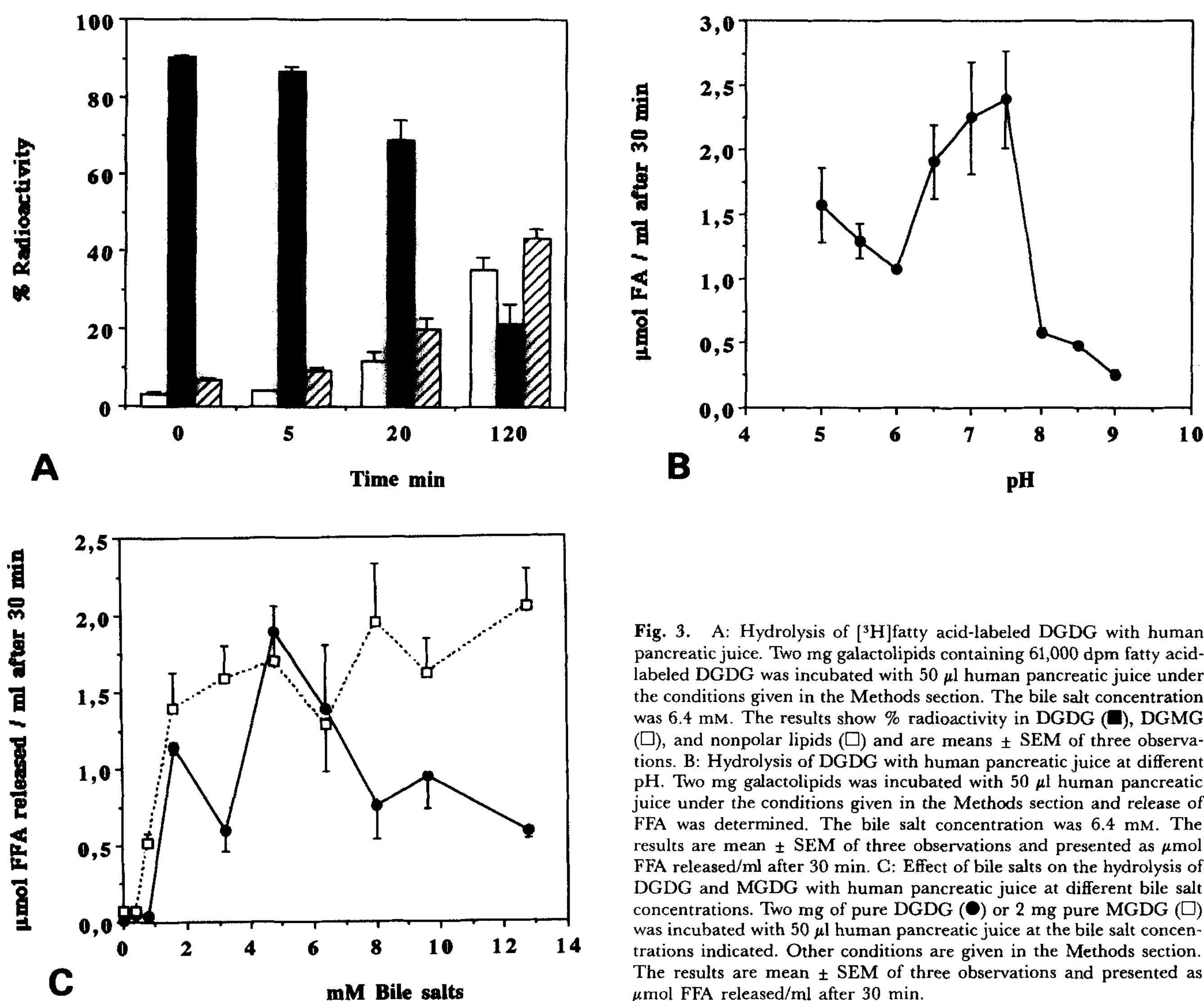

Fig. 3. A: Hydrolysis of [^{3}H]fatty acid-labeled DGDG with human pancreatic juice. Two mg galactolipids containing 61,000 dpm fatty acid-labeled DGDG was incubated with 50 µl human pancreatic juice under the conditions given in the Methods section. The bile salt concentration was 6.4 mM. The results show % radioactivity in DGDG (■), DGMG (□), and nonpolar lipids (□) and are means ± SEM of three observations. B: Hydrolysis of DGDG with human pancreatic juice at different pH. Two mg galactolipids was incubated with 50 µl human pancreatic juice under the conditions given in the Methods section and release of FFA was determined. The bile salt concentration was 6.4 mM. The results are mean ± SEM of three observations and presented as µmol FFA released/ml after 30 min. C: Effect of bile salts on the hydrolysis of DGDG and MGDG with human pancreatic juice at different bile salt concentrations. Two mg of pure DGDG (●) or 2 mg pure MGDG (□) was incubated with 50 µl human pancreatic juice at the bile salt concentrations indicated. Other conditions are given in the Methods section. The results are mean ± SEM of three observations and presented as µmol FFA released/ml after 30 min.

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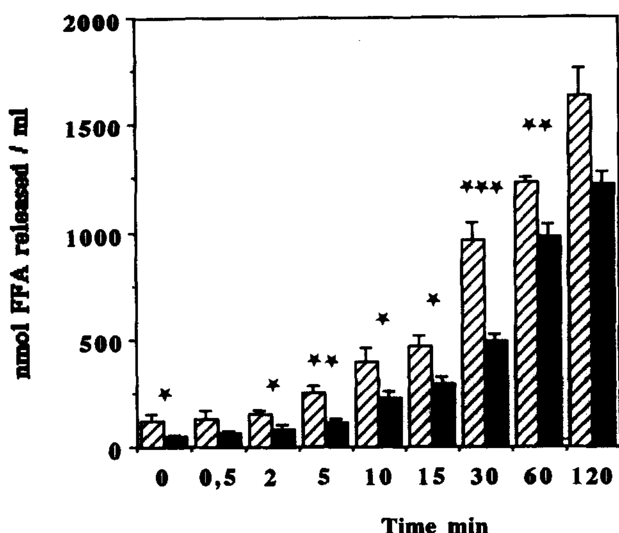

Fig. 4. Effect of antiserum against CEL on the hydrolysis of galactolipids by human pancreatic juice. Two mg galactolipids was incubated with human pancreatic juice under the conditions given in the Methods section with addition of 10 μl antiserum against CEL (■) or 10 μl control serum (□). The bile salt concentration was 6.4 mM. The results are presented as nmol fatty acids released/ml and are means of duplicates.

caused a moderate increase in the lipolysis rate (Fig. 6). Virtually no hydrolysis was observed with 50 μg/ml colipase-dependent lipase without colipase or with colipase concentrations of 10 and 50 μg/ml (molar ratios lipase: colipase 1:1 and 1:5). When pure galactolipid substrates were used, CEL hydrolyzed MGDG, DGDG, and SQDG. The hydrolysis rate was highest for MGDG and slowest for DGDG (**Fig. 7A**). MGDG was also the best substrate for pure Lip-Col (Fig. 7B). This enzyme hydrolyzed SQDG at a slower rate and had negligible activity against DGDG (Fig. 7B). The results thus differed from those obtained with the pancreatic juice which hydrolyzed DGDG as efficiently as MGDG (Fig. 5). The addition of Lip-Col to pure CEL did not increase the hydrolysis rate of DGDG or MGDG. When human pancreatic juice was subjected to chromatography on Sephadex G100 gel, one peak of galactolipase activity comigrated with CEL close to the void volume (**Fig. 8A, 8B**). This peak exhibited activity against both MGDG and DGDG (Fig. 8B). A second galactolipase activity peak that comigrated with the colipase-dependent lipase was identified (Fig. 8A, 8C). In contrast to the purified colipase-dependent lipase the enzyme(s) in this peak was, however, active against all three galactolipids, the activity against DGDG being at least as high as that against MGDG. The lipase activity of this peak was 46 μmol · ml^{-1} · min^{-1}. The amount of lipase in the aliquots found to cause significant hydrolysis of DGDG was thus much lower than the amount of pure colipase-dependent lipase that did not hydrolyze DGDG. As this peak contained considerable amounts of protein, the question was raised whether the presence of protein may stimulate the activity of Lip-Col against DGDG, e.g., by binding released fatty acids. Addition of a similar concentration of albumin did not, however, increase the activity of pure Lip-Col against pure DGDG (data not shown).

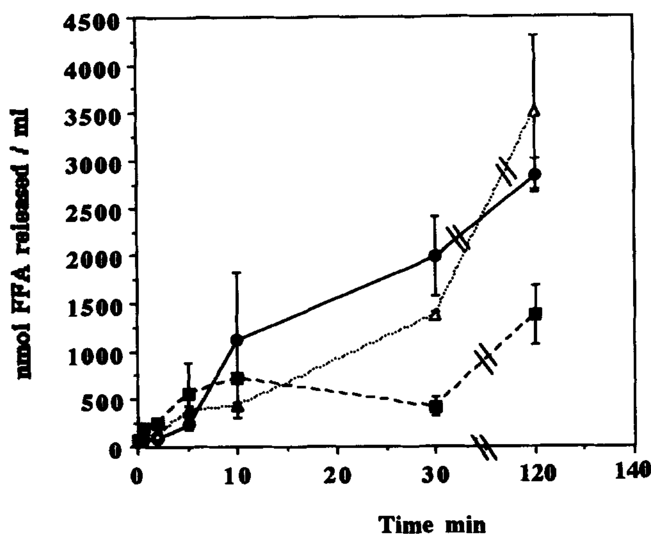

Fig. 5. Hydrolysis of DGDG, MGDG, and SQDG by human pancreatic juice. Two mg of each galactolipid was incubated with 50 μl human pancreatic juice (5%) under the conditions given in the Methods section. The bile salt concentration was 6.4 mM. The results are means of duplicates and presented as nmol FFA released/ml at different time intervals. DGDG (●), MGDG (△), and SQDG (■).

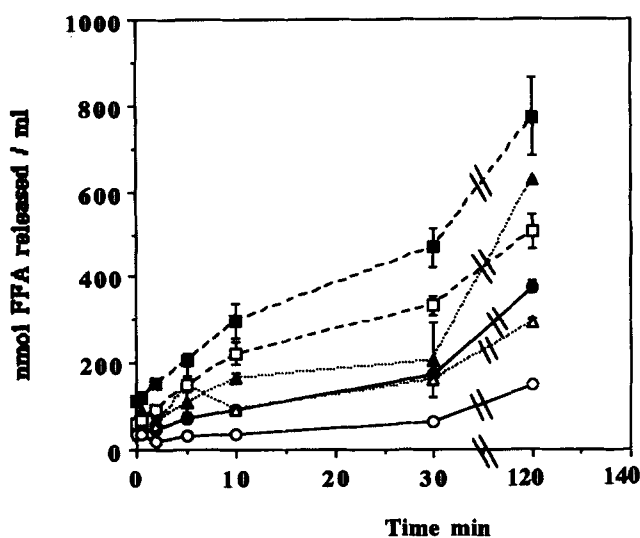

Fig. 6. Hydrolysis of galactolipids by different concentrations of CEL. Two mg galactolipids was incubated with different concentrations of CEL and CEL plus Lip-Col: CEL 10 μg/ml (○), CEL 20 μg/ml (△), CEL 40 μg/ml (□); or with carboxyl ester lipase (CEL) together with colipase-dependent lipase (1:1 w/w) at different concentrations CEL + Lip-Col: 10 + 25 μg/ml (●), 20 + 50 μg/ml *(▲), 40 + 100 μg/ml (■). The bile salt concentration was 6.4 mM. The results are given as nmol fatty acid released/ml incubation and are means ± SEM of three observations (*, only two observations).

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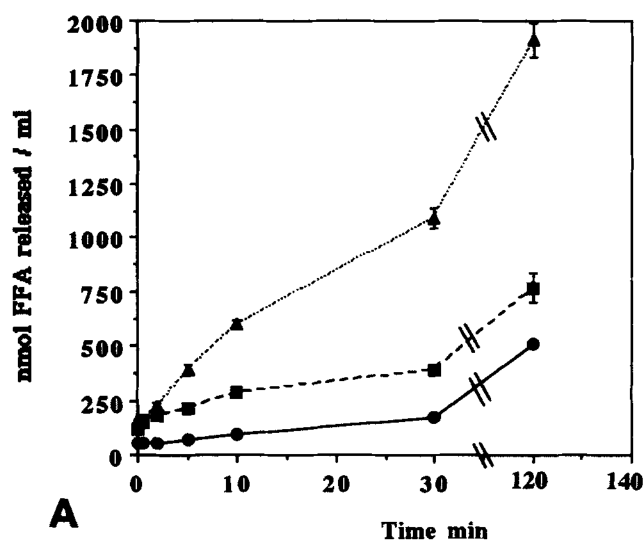


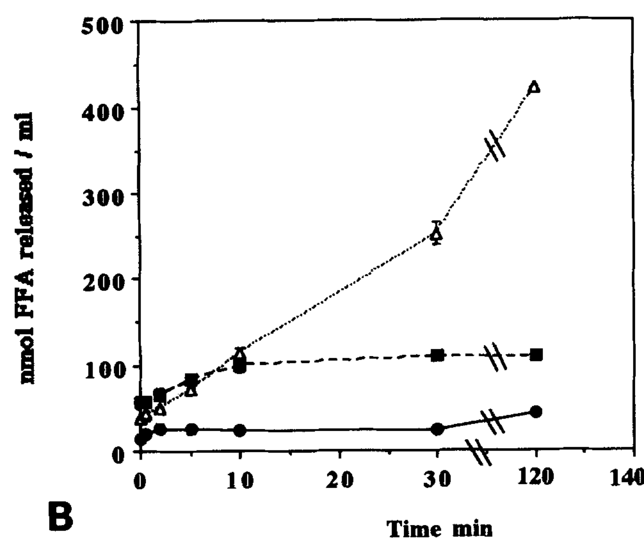
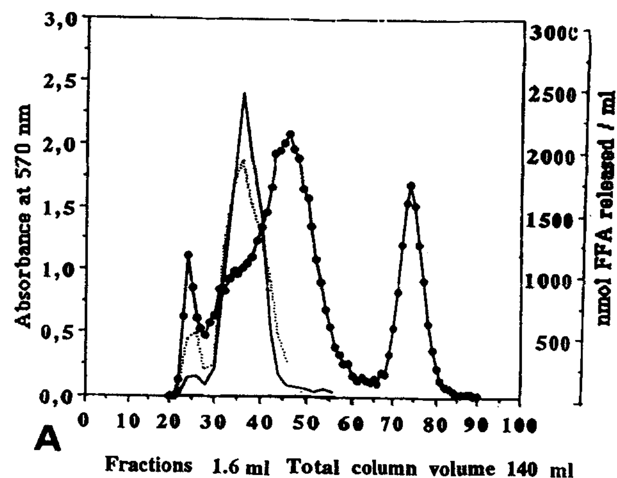


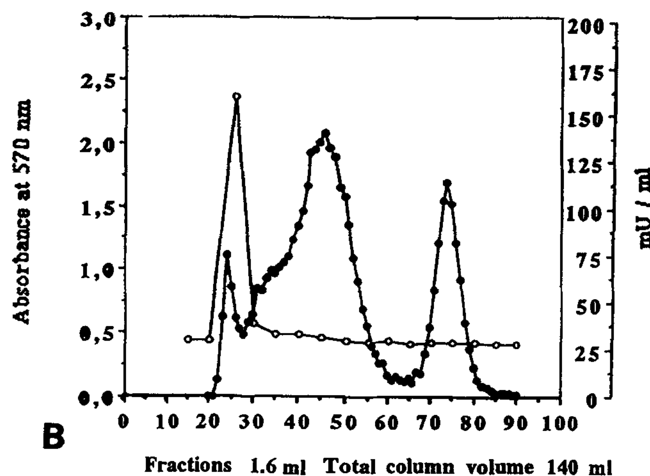
Fig. 7. A: Hydrolysis of DGDG, MGDG, and SQDG by pure CEL. Two mg of either galactolipid was incubated with 30 μg/ml CEL under the conditions described in the Methods section. The bile salt concentration was 6.4 mM. The results are mean ± SEM, n ' 3, and presented as nmol FFA released/ml at different time intervals. DGDG (●), MGDG (▲), and SQDG (■). B: Hydrolysis of DGDG, MGDG, and SQDG by pure Lip-Col. Twenty five μg lipase and 25 μg colipase were incubated with 2 mg pure DGDG, MGDG, or SQDG under the conditions given in the Methods section. The bile salt concentration was 6.4 mM. The results are means ± SEM of three observations. DGDG (8), MGDG (△), and SQDG (■).

Both pure CEL and the Sephadex G100 peak with molecular mass 40–50 kD yielded both DGMG and nonpolar lipids when incubated with [^{3}H]fatty acid labeled DGDG. Phospholipase A_2 (4 μg/ml) did not hydrolyze galactolipids (data not shown).

DISCUSSION

The present study shows that digalactosyldiacylglycerol (DGDG) is hydrolyzed by enzymes in human duodenal





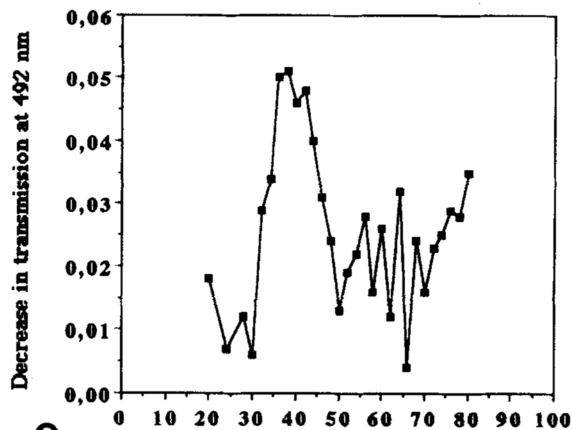


Fig. 8. A: Separation of galactolipidase activities of human pancreatic juice by gel filtration chromatography. Human pancreatic juice was separated on Sephadex G100 as described in the Methods section. Aliquots of the fractions were analyzed for lipolytic activity against DGDG (—) and MGDG (····) and protein content was determined (–●–). The bile salt concentration was 6.4 mM. Other conditions are given in the Methods section. B: CEL activity of gel filtration chromatography fractions of human pancreatic juice. CEL activity was measured as described in the Methods section and is given as $\mu mol \cdot min^{-1} \cdot ml^{-1}$. C: Lipase activity of the gel filtration chromatography fractions of human pancreatic juice. The lipase activity is presented as decrease in transmission of the fractions containing lipase activity, measured as described in the text.

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contents to form free fatty acids, DGMG, and water-soluble compounds. Even during incubation with duodenal contents for up to 2 h, most of the DGMG was not hydrolyzed further. In this respect the digestion of DGDG is thus similar to the hydrolysis of dietary and bile phospholipids by phospholipase A_2 which produces lysocompounds that are well absorbed and further metabolized in the mucosal cells. In our study, phospholipase A_2 was, however, inactive against galactolipids. The formation of DGMG from DGDG thus must be catalyzed by other enzymes.

Enzymes of human pancreatic juice hydrolyzed galactolipids, the rate of hydrolysis of MGDG and DGDG being similar. SQDG was hydrolyzed as well as DGDG and MGDG during the first ten min of incubation but less efficient at later times (Fig. 5). The hydrolysis of galactolipids could be catalyzed by CEL, by Lip-Col, by a concerted action of these enzymes, or by hitherto unknown galactolipase(s) in pancreatic juice. In view of the broad substrate specificity of CEL, a role of this enzyme seemed most likely. The study shows that CEL hydrolyzes both a mixture of galactolipids and pure individual galactolipids, the hydrolysis rate being higher for MGDG than for DGDG and SQDG (Fig. 7A). Formation of DGMG from incubation of [^{3}H]fatty acid-labeled DGDG could also be demonstrated with both pancreatic juice (Fig. 3A) and with pure CEL. Furthermore, antiserum against CEL caused a partial inhibition of the hydrolysis of the mixed galactolipids with pancreatic juice (Fig. 4). The conclusion is that CEL participates in the digestion of all three major galactolipids, but its quantitative role has not been defined.

Pure Lip-Col catalyzed some hydrolysis when incubated with the galactolipid mixture (data not shown), and also increased the hydrolysis when added together with CEL (Fig. 6). Pure Lip-Col hydrolyzed pure MGDG and, at a slow rate, SQDG, but had very little effect on DGDG under the incubation conditions used. Both CEL and Lip-Col thus hydrolyzed MGDG better than DGDG (Fig. 7A and B), in contrast to human pancreatic juice (Fig. 5). This raised the question whether another enzyme(s) in human pancreatic juice may possess activity against DGDG. When proteins of human pancreatic juice were separated by gel filtration on Sephadex G100, two peaks of galactolipase activity were demonstrated, one comigrating with CEL close to the void volume, and one with a molecular mass of 40-50 kDa which comigrated with Lip-Col (Fig. 8). Like pure CEL, the first peak hydrolyzed MGDG better than DGDG. In contrast to the pure Lip-Col which had no or little activity against DGDG, the second peak hydrolyzed, DGDG and MGDG at similar rates (Fig. 8A, B). The data are thus compatible with the hypothesis that an enzyme in pancreatic juice that is not identical to the colipase-dependent lipase but which has a similar molecular mass is active against DGDG. Recently, the existence of two proteins in pancreatic juice with a high degree of homology with colipase-dependent lipase (PLRP 1 and PLRP 2) was demonstrated (23, 24). The possibility that either of these proteins is a galactolipase with high activity against DGDG should be examined.

Conclusions concerning the quantitative role of different lipases in vivo must also await further studies using galactolipids in different physical form. Thus, galactolipids might be more efficiently hydrolyzed by the colipase-dependent lipase when they are mixed with more hydrophobic dietary lipids, which Lip-Col may act on at an oil-water interface.

In summary, the study shows that enzymes of the pancreatic juice catalyze a bile salt-dependent hydrolysis of both MGDG and DGDG. The hydrolysis is in part catalyzed by CEL and in part by an enzyme with a molecular weight similar to the colipase-dependent lipase. Further studies should be focused on the identity of this enzyme and on the effects of the physical form of the substrate on the hydrolysis of galactolipids. Furthermore, the fate of the DGMG during absorption should be studied and the products formed during digestion of MGDG and SQDG should be characterized.

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