Interconversion of α-linolenic acid in rat intestinal mucosa: studies in vivo and in isolated villus and crypt cells

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Abstract The desaturation-elongation of [^{14}C]linolenic acid (n-3) in the rat small intestine was examined in homogenates of villus and crypt cells in vitro and in mesenteric lymph duct cannulated rats in vivo, using high performance liquid chromatography of fatty acid methyl esters with continuous flow measurement of ^{14}C. Desaturation-elongation of [^{14}C]18:3 to [^{14}C]18:4, [^{14}C]20:4, and [^{14}C]20:5 (n-3) could be demonstrated in both villus and crypt cell homogenates, the rate of interconversion generally being highest in the villus cell fraction. For example, during incubation with 20 nmol [^{14}C]linolenic acid (n-3), the average rates of Δ6 desaturation were 1.35 pmol/min per mg protein in villus and 0.93 pmol/min per mg protein in crypt cell homogenates. The acylation of [^{14}C]linolenic acid (n-3) into phospholipids in crypt cells was twofold higher than in villus cells. Addition of 1-palmitoyl-*sn*-glycero-3-phosphocholine (20-240 μM) increased the incorporation of [^{14}C]linolenic acid (n-3) into phosphatidylcholine, and decreased the interconversion of [^{14}C]linolenic acid (n-3) to Δ6 and Δ5 desaturation products in both villus and crypt cells. 1-Palmitoyl-*sn*-glycero-3-phosphocholine also decreased the incorporation of ^{14}C into triacylglycerols and phosphatidic acid, the decrease being more pronounced in the crypt cells. At a constant concentration of 20 μM [^{14}C]linolenic acid (n-3), estimated Michaelis constants (K_m) and maximal velocities (V_{max}) of the linolenoyl-CoA:1-palmitoyl-*sn*-glycero-3-phosphocholine acyltransferase activity for 1-palmitoyl-*sn*-glycero-3-phosphocholine was 111.1 μM 1-palmitoyl-*sn*-glycerol-3-phosphocholine and 0.45 pmol/min per mg protein in crypt cells and 571.4 μM and 1.09 pmol in villus cells, respectively. When [^{14}C]linolenic acid (n-3) was fed to mesenteric duct-cannulated rats in a linoleate-rich soybean lipid emulsion (Intralipid), there was no measurable ^{14}C in desaturation products in lipids of the small intestinal mucosa after 4 h, or in chyle collected during 4 h. When the rats were infused with an emulsion containing [^{14}C]linolenic acid (n-3) and 5 mg trilinolenin, 0.52 ± 0.37% (mean ± SEM) of the ^{14}C radioactivity in chyle was in eicosapentaenoic acid (n-3). The data thus indicate that Δ6 and Δ5 desaturase are present in both villus and crypt cells. However, the enzymes metabolize only a small amount of the dietary linolenic acid (n-3). This may be due to the rapid acylation into phospholipids and triacylglycerol during its absorption and incorporation into chylomicrons. The high activity of acyl-CoA:1-acyl-*sn*-glycero-3-phosphocholine acyltransferase in crypt cells may favor the retention of polyunsaturated fatty acids in phospholipids during cell regeneration and maturation, but may also be a limiting factor for the rate of fatty acid interconversion.—**Chen, Q., and A. Nilsson.** Interconversion of α-linolenic acid in rat intestinal mucosa: studies in vivo and in isolated villus and crypt cells. *J. Lipid Res.* 1994. **35:** 601-609.

Supplementary key words acyl CoA:1-acyl-*sn*-glycero-3-phosphocholine acyltransferase • desaturation • elongation • eicosapentaenoic acid • phosphatidylcholine • reacylation • small intestine • triacylglycerol

Long chain polyunsaturated fatty acids are important structural components of enterocyte membrane phospholipids (1). These fatty acids are also continuously required to form the phospholipid surface coat of chylomicrons during fat absorption (2, 3) and are substrates for the synthesis of eicosanoids in mucosal cells. The polyunsaturated fatty acids in the small intestinal tract are in part derived from dietary sources (4) and from fatty acids released from bile phospholipids by the action of the pancreatic phospholipase A_2 (5).

Recent studies indicate that the enterocytes of the small intestine may desaturate and elongate essential fatty acids, the rate limiting enzyme, Δ6 desaturase activity of the enterocytes, being associated with the microsomal membranes as in hepatocytes and brain cells (6, 7). High activities of Δ6 desaturase-elongase and Δ5 desaturase-elongase were also observed in the human CaCo-2 cell line, which has several structural and biochemical properties of enterocytes (8). In a recent study, we observed that intravenously injected albumin-bound [^{14}C]linolenic acid

Abbreviations: GPC, glycero-3-phosphocholine; HPLC, high performance liquid chromatography; PA, phosphatidic acid; PAF-18, platelet activating factor-18 (1-O-octadecyl-2-O-methyl-*sn*-glycerophosphocholine); PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triacylglycerol; 18:2(n-6), linoleic acid; 18:3(n-3), α-linolenic acid; 20:4(n-6), arachidonic acid; 20:5(n-3), eicosapentaenoic acid; TLC, thin-layer chromatography.

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(18:3, n-3) was converted to [^{14}C]eicosapentaenoic acid (20:5, n-3) in stomach, small intestine, and colon (Å. Nilsson and W. Becker, unpublished data). These findings suggest that a certain proportion of the body's C-20 and C-22 polyunsaturated fatty acids may originate by chain elongation-desaturation of linolenic acid (18:2, n-6) and 18:3 in the small intestine. However, the distribution of these enzymes among different cell types in the gastrointestinal tract and their quantitative role in vivo have not been characterized.

The present study was undertaken to get further information about the ability of the intestinal mucosal cells at different stages of maturation to synthesize their own eicosanoid precursor pools. As 18:3 is a preferred substrate for the Δ6 desaturase that catalyzes the initial rate limiting step also in the interconversion of 18:2 to arachidonic acid (20:4, n-6), we used [^{14}C]18:3 as substrate. The distribution of activity of desaturase-elongase and acyl-CoA:1-acyl-glycero-phosphocholine (GPC) acyltransferase between villus and crypt cells of small intestinal cells was examined. We also studied whether any dietary 18:3 underwent interconversion before being incorporated into chyle lipids in experiments where [^{14}C]18:3 (n-3) was fed to mesenteric duct-cannulated rats.

MATERIAL AND METHODS

Material

[1-^{14}C]18:3 (51.0 mCi/mol) was purchased from New England Nuclear (Boston, MA). Unlabeled polyunsaturated fatty acids, authentic fatty acid standards for high performance liquid chromatography (HPLC), and 1-palmitoyl-glycerol-GPC were obtained from Sigma (St. Louis, MO) or from Nu-Chek Prep (Elysian, MN). 1-O-octadecyl-2-O-methyl-*sn*-glycero-3-phosphocholine (PAF-18) is a product of Cayman Chemical Company (Ann Arbor, MI). All other chemicals used were analytical grade.

Experiments in vitro

Preparation of villus and crypt cell homogenates. The sequential isolation of villus from crypt cells was performed according to the method of Weiser (9) as modified by Bjerknes and Cheng (10). Non-fasted male white Sprague-Dawley rats (ALAB, Stockholm, Sweden), weighing 200-250 g, were used. Forty cm of proximal small intestine was removed immediately after decapitating the rats and was washed with an ice-cold solution containing 96 mM NaCl, 27 mM sodium citrate, 1.5 mM KCl, 0.8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, 10 mg/l gentamicine, and 0.5 mM dithiothreitol (DTT), pH 7.4 (solution A). The intestine was filled with 20 ml solution A and incubated for 10 min at 37°C. This solution was then discarded as it contained primarily mucus, bacteria, and other luminal contents. The intestine was then filled with a buffer containing 0.3 mM EDTA, 115 mM NaCl, 25 mM $NaHCO_3$, 2.4 mM K_2HPO_4, 2.5 mM L-glutamine, 0.5 mM B-hydroxybutyrate, and 0.5 mM DTT, pH 7.4 (solution B). It was incubated in solution B for 3 min at 37°C and was gently palpated to facilitate cell dispersion. This solution was collected in plastic conical centrifuge tubes and contained villus tip cells as fraction one. Similarly, five more cell fractions were sequentially collected, with fraction six containing cells from the crypt region. The fractions were then washed free of solution B with modified L-15 medium by centrifuging twice at 1,500 *g* for 5 min. Alkaline phosphatase activity was used as a marker of villus cells and thymidine kinase activity as a marker of crypt cells. Alkaline phosphatase was determined using a standard kit purchased from Sigma Chemical Co., St. Louis, MO. Thymidine incorporation into DNA was determined as an index of thymidine kinase activity (11). Each cell fraction was incubated with 5-7 μCi of [methyl-^{3}H]thymidine in 5 ml L-15 medium containing 5-10 mg cell protein for 2 h at 37°C. Crude DNA extraction was made by homogenizing the cells and adding an equal volume of 1 N perchloric acid. Samples were then solubilized in 0.1 N NaOH and aliquots (0.5 ml) were added to scintillation vials with 0.5 ml methanol and 10 ml scintillation fluid (Instagel-toluene, 1:1). Radioactivity was determined in a Packard 460 CD liquid scintillation system with external quench correction. In initial experiments, viability of cells was assessed by trypan blue exclusion and was found to be at least 90%. Cell fractions were suspended in a 0.25 M sucrose buffer containing 5 mM MgCl, 0.15 M KCl, 1.5 mM glutathione, 50 mM KH_2PO_4, pH 7.2, at 4°C, and were then homogenized on ice by a Branson Sonifier 250 (two times 30 sec in 10 watt). Protein content in each cell fraction was measured by the Lowry method using a Sigma Protein Assay Kit, indicating approximately equal percentage of total cell protein.

Microsomal preparation. Microsomes from villus and crypt cells were prepared according to the method of Garg et al. (7). The sonicated homogenates were centrifuged at 600 *g* for 10 min to remove unbroken cells and nuclei. The supernatant was centrifuged at 12,000 *g* for 15 min to pellet the mitochondrial fraction. The 12,000-*g* supernatant was then centrifuged at 105,000 *g* for 60 min to obtain the microsomal pellet. The washed microsomal pellet was resuspended in 0.25 M sucrose buffer containing 0.15 M KCl (pH 7.4) to a concentration of approx. 10 mg microsomal protein per ml for enzyme assay.

Determination of interconversion and acylation. To determine the rate of interconversion in villus and crypt cell homogenates and in microsomal preparations, [^{14}C]18:3 was used as substrate. The labeled fatty acid was bound to bovine serum albumin (essential fatty acid-free, Sigma) and the assay was performed as described earlier (8). The

assay mixture contained (in a final volume of 1.5 ml) 5 μmol ATP, 0.1 μmol CoA-SH, 0.5 μmol niacinamide, 1.25 μmol NADH, 2.25 μmol glutathione, 5 μmol $MgCl_2$, 62.5 μmol NaF, 62.5 μmol KH_2PO_4, pH 7.4, and 19 nmol/μCi (or as indicated) of albumin bound [^{14}C]18:3. The reaction was started by adding 0.2-ml aliquots of homogenates containing 5–10 mg protein. The samples were incubated for 20 min at 37°C in a shaking water bath. The reaction was stopped by adding 8 ml chloroform–methanol 1:2 (v/v) containing 0.005% (w/v) butylated hydroxytoluene (BHT). Acyl CoA:1-acyl-GPC acyltransferase activity in homogenates of villus and crypt cells was measured as follows. Aliquots of 1-palmitoyl-GPC in chloroform solution were dried with nitrogen in test tubes and other components of the assay mixture described above, including albumin-bound [^{14}C]18:3 were then added. The reaction was initiated by adding aliquots of the homogenates to a total volume of 1.5 ml. In some experiments, attempts were made to inhibit the acylation of 18:3 into phospholipids and thereby increase the interconversion by adding PAF-18, which has been shown to inhibit acylation of added 1-acyl-GPC in different cell types (12). The PAF-18 was dissolved in 0.9% saline and added as final concentrations as indicated in Results.

Experiments in vivo

Male white Sprague-Dawley rats, weighing 200–250 g, fed a commercial standard pellet diet, were used. The rats were kept fasting and had free access to tap water a day before operation. Mesenteric duct cannulations were performed according to Warshaw (13) and the rats were postoperatively treated as described earlier (14). Radioactive lipid emulsion was prepared as follows: 20–25 μCi of [^{14}C]18:3 as an ethanol solution was mixed with 0.5 mg egg phosphatidylcholine in 0.1% chloroform solution and dried with nitrogen. The dry residue was immediately dispersed with 1 ml of hot physiological saline. The radioactive mixture was then added to either 2 ml 20% Intralipid or 1 ml 1% gum arabic solution which had been sonicated with 5 mg pure trilinolenin on ice for three × 30 sec. two ml of either labeled lipid emulsion was then infused through the gastric fistula over 1 h as indicated. The radioactive chyle was collected on ice in the presence of 2 mM EDTA (final concentration) and stored under nitrogen at 4°C before analysis of lipids. Rats were decapitated after 4 h. Approximately 20 cm of each of the proximal (jejunum) and distal (ileum) small intestine was removed and immersed in ice-cold physiological saline to remove luminal contents. Segments were placed on a prechilled glass plate, opened longitudinally along the mesenteric border, and gently blotted to remove excess moisture. The mucosal surface was removed by gently scraping with a microscope slide and was placed in preweighed tubes containing 5 ml chloroform–methanol 1:1 (v/v) for lipid extraction as described below.

Lipid analysis

Lipids were extracted as described earlier (8). After two-phase distribution, the chloroform phase was dried under nitrogen and the residue was redissolved in 1.0 ml chloroform. The proportion of ^{14}C present in interconversion products of [^{14}C]18:3 was estimated by HPLC of fatty acid methyl esters linked to continuous flow measurement of ^{14}C. Aliquots of the lipid extracts were transmethylated as follows. The extract was taken to dryness with nitrogen; the residue was redissolved in 1 ml toluene–methanol 1:1 (v/v) containing 2% H_2SO_4, and heated at 65°C for 4 h; 0.5 ml H_2O and 1.5 ml of petroleum ether were then added. The upper phase was collected and the lower phase was washed twice with 0.5 ml petroleum ether. Aliquots of fatty acid methyl esters in the petroleum ether were taken to dryness and redissolved in 20 μl acetonitrile–water 90:10 (v/v). The labeled PUFA methyl esters were then analyzed by HPLC using a Shimadzu LC 6A apparatus equipped a Nucleosil C18 column (5 μm, 250 × 4.6 mm) that was eluted by acetonitrile–water 90:10. The flow rate was 0.5 ml/min and the absorbance was monitored with a Shimadzu SPD 6A UV detector at 205 nm. Peaks were identified by comparing retention times with authentic fatty acid methyl ester standards. The radioactivity was determined by continuous flow liquid scintillation counting using a Radiomatic Flow-One β radioactivity flow detector, with channels setting at 25 to 100 for ^{14}C radioactivity. The detector was operated with Flo-Scint II scintillation fluid (Radiomatic Instrument and Chemical, Co., Tampa, FL) at a flow rate of 2.5 ml/min using a 0.5 ml cell.

To determine the distribution of radioactivity between different lipid classes, lipids were separated by thin layer chromatography (TLC). Aliquots from the extracts were separated on silica gel G plates that were developed in light petroleum–diethylether–acetic acid 80:20:1 (v/v/v) for neutral lipid separations and in chloroform–methanol –acetic acid–water 100:80:12:1.2 (v/v/v/v) for polar lipid separation. Spots were visualized by iodine vapor and scraped into counting vials. One ml methanol–water 1:1 (v/v) and 9 ml toluene–Instagel 1:1 (v/v) were added and radioactivity was determined in a Packard 460 CD liquid scintillation system. Quenching was determined using an external standard.

RESULTS

Preparation of villus and crypt cells

The characterization of the different cell fractions by alkaline phosphatase and thymidine kinase measurements is shown in **Fig. 1.** Fractions 1–3 contained high alkaline phosphatase and only low thymidine kinase activity, indicating that they contained mainly villus cells. In contrast, fraction 6 contained high thymidine kinase and very

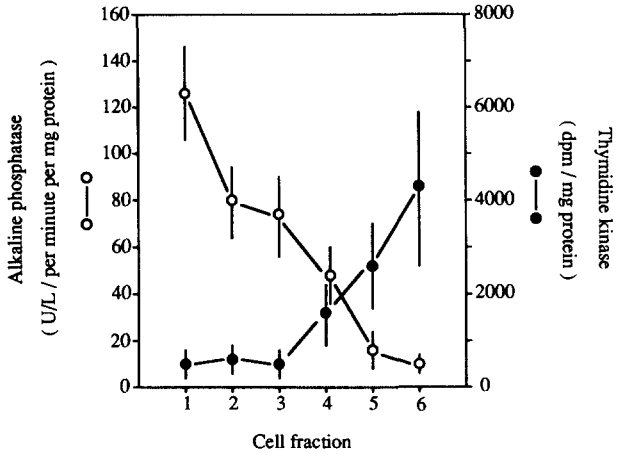

Fig. 1. Marker enzyme activities in different cell fractions. Alkaline phosphatase activity is expressed as U/L per min per milligram of protein and thymidine kinase activity for each fraction is expressed as dpm per milligram protein. The protein contents in fractions 1 and 6 from three separate cell preparations were 33.5 ± 1.6 mg and 36.2 ± 2.3 mg, respectively. Similar amounts of protein were obtained in each of fractions 2-5. Results are means ± SEM for six determinations from three preparations.

low alkaline phosphatase activity, indicating that it was mainly crypt cells. Fraction 4 contained both enzymes. This indicates that there was a sequential removal of enterocytes from the villus tip to the crypts as previously demonstrated by Weiser (9). The different types of cells were then used for preparation of homogenates or subcellular fractions.

Interconversion rate of [^{14}C]18:3 in cell homogenates from various levels of the villus-crypt axis

In this series of experiments, Δ6 and Δ5 desaturation was determined in the homogenates prepared from the six different cell fractions. The rate of Δ6 desaturation in this study was expressed as pmol [^{14}C]18:3 that had been converted to [^{14}C]18:4 (n-3), [^{14}C]20:4(n-3), and [^{14}C]20:5(n-3), per min/mg protein. Δ5 Desaturation was expressed as pmol [^{14}C]18:3 that had been converted to [^{14}C]20:5. As shown in **Table 1,** there was a decline in rates of interconversion along the villus-crypt cells axis, i.e., the interconversion rate was highest in fractions 1-4 (mainly villus) and lowest in fraction 6 (crypt). Effects of substrate concentration on enzyme activity were observed by using three concentrations of [^{14}C]18:3 (2, 20, and 200 nmol per incubation, **Table 2**). In this concentration range, saturation kinetics was not achieved, but the reaction rates were influenced by substrate concentration in a similar way in villus and crypt cell homogenates. The rate of interconversion of 18:3 was higher in villus cells (fraction 1) than in crypt cells (fraction 6).

TABLE 1. Rates of interconversion of [^{14}C]18:3 in incubations with cell homogenates

Cell Fraction	Interconversion Rate	
	Δ6 Desaturation	Δ5 Desaturation
	pmol/min/mg protein	
Villus		
1	3.12	1.70
2	2.70	1.68
3	3.38	1.88
4	2.93	1.75
5	1.85	1.06
Crypt 6	0.98	0.64

The incubations were initiated by adding cell homogenates containing 5 mg protein from different cell fractions in the presence of 19 nmol albumin-bound 18:3 containing 1 μCi ^{14}C. Other incubation conditions were described in Methods. Results are means of duplicates from one of two similar experiments. Δ6 Desaturation includes formation of [^{14}C]18:4, 20:4, and 20:5, whereas Δ5 desaturation is the rate of formation of [^{14}C]20:5 only.

Interconversion in subcellular fractions

In this series of experiments, interconversion and acylation of [^{14}C]18:3 in microsomes and in 12,000-*g* pellets containing the mitochondrial fraction from both villus (fraction 1) and crypt (fraction 6) cells were examined. **Fig. 2** compares the formation of [^{14}C]18:4, [^{14}C]20:4, and [^{14}C]20:5 after incubation with microsomes or 12,000-*g* pellets. Desaturation and elongation occurred at similar rates both in microsomes and in 12,000-*g* pellets.

TABLE 2. Effects of substrate concentration on the interconversion of [^{14}C]18:3 in the incubations with villus and crypt cell homogenates

Substrate Conc.	Interconversion Rate	
	Δ6 Desaturation	Δ5 Desaturation
	pmol/min/mg protein	
Villus		
2 nmol	0.30 ± 0.06	0.14 ± 0.02[b]
20 nmol	1.35 ± 0.15[b]	0.78 ± 0.07[a]
200 nmol	7.54 ± 1.00	4.02 ± 0.42
Crypt		
2 nmol	0.22 ± 0.09	0.07 ± 0.02
20 nmol	0.93 ± 0.13	0.40 ± 0.04
200 nmol	6.60 ± 0.87	3.22 ± 0.17

Cell homogenates of villus and crypt fractions were incubated in the presence of three concentrations of [^{14}C]18:3. The incubations were started by adding cell homogenates containing 5 mg protein. For other incubation conditions see Methods. Results are mean ± SEM of eight incubations from three animals.

[a] $P < 0.01$, [b] $P < 0.05$ vs. incubations with crypt homogenates at the same concentration of 18:3, respectively.

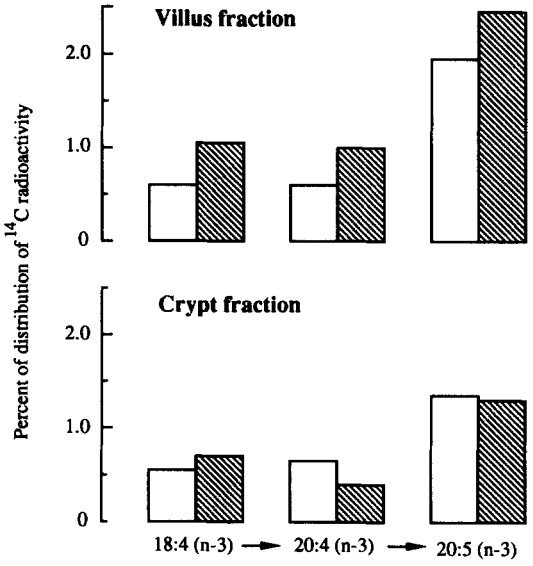

Fig. 2. Interconversion of [^{14}C]18:3 with microsomes and 12,000-*g* pellets from villus and crypt cells. The rates of interconversion in microsomes (open bars) and 12,000 *g* pellets (filled bars) are shown as percent distribution of ^{14}C radioactivity in desaturating-elongating products of [^{14}C]18:3. Twelve nmol of [^{14}C]18:3 was added to each incubation. Other incubation conditions see Materials and Methods. Results are means of two determinations from one of two separate cell preparations.

Acylation of [^{14}C]18:3 into phospholipids and triacylglycerol

The rate of acylation of ^{14}C-labeled fatty acids and the distribution of esterified fatty acids among different phospholipid classes was assayed by incubating homogenates from the six different cell fractions with [^{14}C]18:3. Without addition of 1-palmitoyl-GPC 4.8–16.0 pmol of ^{14}C-radioactivity per min/mg protein was incorporated into PA, 0.9–2.2 pmol per min/mg into PE, and 1.4–5.2 pmol per min/mg protein into PC (**Table 3**). The rate of acylation of radioactive fatty acids into PC and PE was highest in the crypt cells, i.e., in the fractions in which the degree of desaturation and elongation was lowest. Incorporation into PA was highest in fraction 4, but was higher in cell fractions 1–4 than in fractions 1 and 2. The rates of incorporation of [^{14}C]18:3 into TG were similar in all six cell fractions (Table 3).

TABLE 3. Incorporation of [^{14}C]18:3 into phospholipids and triacylglycerol in incubations with cell homogenates from different cell fractions

	Rate of Incorporation			
Cell Fraction	TG	PA	PE	PC
	pmol/min/mg protein			
Villus				
1	47.35	4.77	0.94	1.43
2	42.91	5.97	0.89	1.29
3	41.38	9.37	1.08	1.58
4	44.57	16.04	1.44	1.63
5	45.85	11.82	1.81	3.68
Crypt 6	41.61	10.69	2.23	5.19

The data were obtained from the incubations described in the Table 1. Data are expressed as means of duplicates from one of two similar experiments.

Effects of 1-palmitoyl-GPC on interconversion and acylation reactions

To examine whether the rate of interconversion of [^{14}C]18:3 was influenced by the rate of acylation of fatty acids into phospholipids and TG in villus and crypt cells, acylation and interconversion were examined in the presence of increasing concentrations of 1-palmitoyl-GPC during incubation. **Fig. 3** shows the effects of 1-palmitoyl-GPC on the formation of radioactive PC when homogenates of villus and crypt cells were incubated with [^{14}C]18:3 and cofactors. The formation of ^{14}C-labeled PC with crypt cell homogenates exceeded that with villus cell homogenates at concentrations below 80 μM 1-palmitoyl-

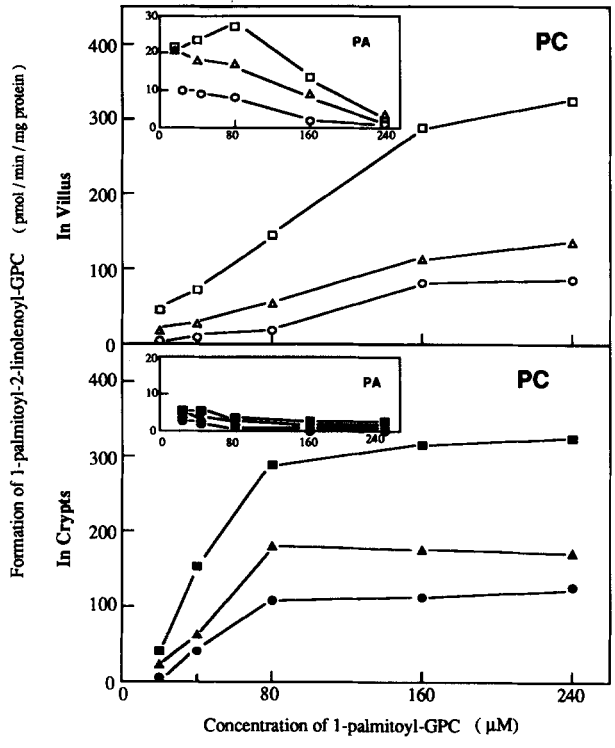

Fig. 3. Effects of 1-palmitoyl-GPC on incorporation of [^{14}C]18:3 into PC and PA in homogenates of villus and crypt cells. The incubations were performed with three concentrations of labeled 18:3 and varying concentrations of 1-palmitoyl-GPC as acyl acceptor. Squares represent 20 μM of [^{14}C]18:3, triangles 10 μM of [^{14}C]18:3, and circles 5 μM of [^{14}C]18:3. The left upper panels in the figures show the effect of 1-palmitoyl-GPC on the incorporation of ^{14}C into PA. For other incubation conditions see Materials and Methods. Data were means of duplicates from one of two similar experiments.

GPC. At the highest concentration, 240 μM, there was no difference between the villus and crypt cell homogenates. The values for the incorporation of ^{14}C into PC using increasing concentrations of 1-palmitoyl-GPC and a constant concentration of [^{14}C]18:3 (20 μM) conformed to linear double reciprocal plots, from which apparent Michaelis-Menten constants (K_m) and maximal velocities (V_{max}) could be calculated (**Fig. 4**). K_m was 571.4 μM 1-palmitoyl-GPC in villus and 111.1 μM in crypt cell homogenates. V_{max} was 1.09 and 0.45 pmol/min per mg, respectively. At 1-palmitoyl-GPC concentrations below 80 μmol, the incorporation of ^{14}C into PA and into TG was much higher in the villus than in the crypt cell homogenates. At concentrations above 80 μM the incorporation into PA and TG was suppressed also in the villus cell homogenates Fig. 3 and **Fig. 5**). A strong suppression of the desaturation-elongation of [^{14}C]18:3 was observed when increasing concentrations of 1-palmitoyl-GPC were added to the incubations with villus cell homogenates (**Fig. 6**). 1-Palmitoyl-GPC suppressed the interconversion moderately in the incubations with crypt cell homogenates. Even the basal level of interconversion was lower in the crypt fraction.

Effects of PAF-18 on acylation and interconversion of [^{14}C]18:3

The higher rate of phospholipid acylation combined with a lower rate of interconversion in the crypt than in the villus cell homogenates suggested that the acylation and the interconversion reactions may compete for the same pool of [^{14}C]18:3-CoA. Experiments were therefore performed in which PAF-18, which was shown to inhibit acyl CoA:1-acyl-GPC acyltransferase in other cell types (12), was added. The purpose was to see whether an inhibition of this enzyme was linked to an increased interconversion of 18:3. A high concentration of PAF-18 (160 μM) inhibited, however, the incorporation of ^{14}C not only into PC, but also into PE, PA, and TG in incubations with villus or crypt cell homogenates (data not shown). Ten-80 μM PAF-18 had only small or no effects on the acylation and interconversion reactions. PAF-18 at 160 μM decreased both the incorporation of [^{14}C]18:3 into PE, PC, and TG and the interconversion of [^{14}C]18:3 to its higher homologues (data not shown). PAF-18 could thus not be used as a means to regulate the partitioning of [^{14}C]18:3 between phospholipid acylation and interconversion.

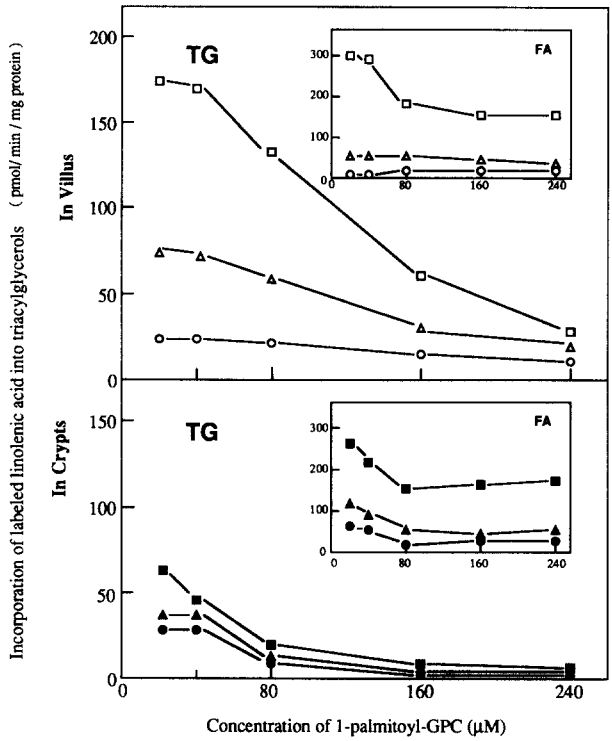

Fig. 5. Effects of 1-palmitoyl-GPC on the incorporation of [^{14}C]18:3 into triacylglycerols in homogenates of villus and crypts. The incubations were performed with three concentrations of labeled 18:3 as exogenous linolenoyl donor and varying concentrations of 1-palmitoyl-GPC. Squares represent 20 μM of [^{14}C]18:3, triangles 10 μM of [^{14}C]18:3, and circles 5 μM of [^{14}C]18:3. The right upper panels in the figures show remaining free fatty acids that were not esterified to PLs and TG after 20 min incubation. Other assay conditions see Materials and Methods. Data were means of duplicates from one of two separate experiments.

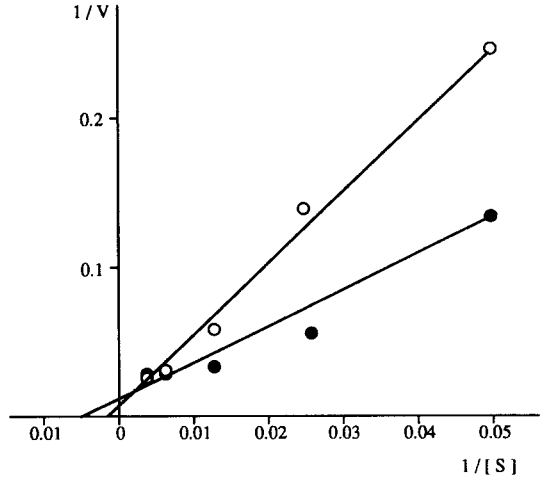

Fig. 4. Michaelis-Menten kinetics for incorporation of 18:3 into PC as a function of 1-palmitoyl-GPC concentration in homogenates of villus and crypt cells. Initial velocities for the rate of esterification of [^{14}C]18:3 into PC as a function of the 1-palmitoyl-GPC concentration in incubation with villus homogenates (open circles) and with crypt homogenates (filled circles). The concentration of [^{14}C]18:3 was 20 μM. For other assay conditions see Materials and Methods. Values are means of two to three separate experiments in duplicate. Lineweaver-Burk plots: S = μM 1-palmitoyl-GPC; V = pmol of 18:3 incorporated into PC per min per mg protein.

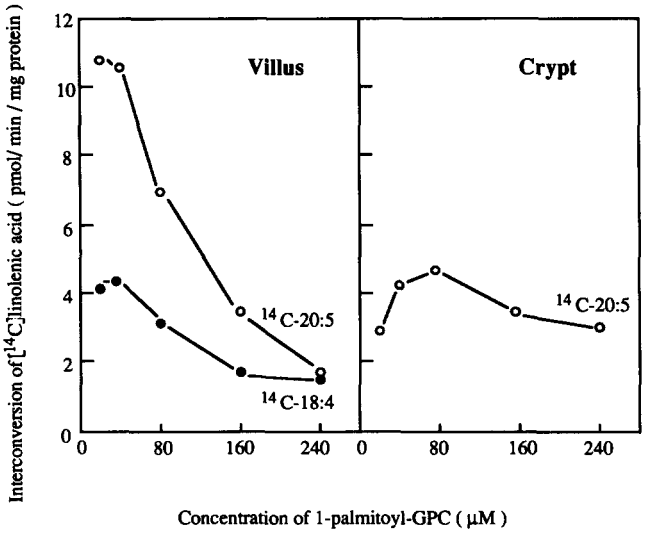

Fig. 6. Effects of 1-palmitoyl-GPC on the interconversion of [^{14}C]linolenic acid into [^{14}C]octadecatetraenoic acid and [^{14}C]eicosapentaenoic acid in homogenates of villus and crypt cells. The incubation mixture contained 20 μM of [^{14}C]18:3 and increasing concentrations of 1-palmitoyl-GPC. There was no measurable formation of radioactive octadecatetraenoic acid in the incubations with crypt cell homogenates. For assay conditions see Materials and Methods. Data were means of two separate experiments, including duplicate determinations.

Desaturation and elongation of [^{14}C]18:3 in in vivo experiments

To examine whether any interconversion of 18:3 occurred during fat absorption in vivo, rats were infused introduodenally for 2 h with [^{14}C]18:3 mixed either with Intralipid or with an emulsion of 5 mg trilinolenin stabilized with gum arabic. Chyle was collected and mucosal scrapings from jejunum and ileum were also obtained after killing the rats, i.e., after 4 h. With Intralipid as a fat vehicle, no radioactive desaturation-elongation products were found either in mucosal scrapings or in the collected chyle. 86.0% and 88.6% of the [^{14}C]18:3 was in TG in jejunal and ileal mucosal scrapings, respectively, and 4.5% and 5.5% in phospholipids. In the chyle, 95.4% of [^{14}C]18:3 was in TG and only 1.3% in phospholipids (**Table 4A**). In the experiments, in which the animal received [^{14}C]18:3 dispersed with 5 mg trilinolenin, relatively higher proportions of radioactive fatty acids were incorporated into phospholipids than TG in both jejunal and ileal mucosal scrapings. Three.six percent of ^{14}C radioactivity in chyle was found in phospholipids and 82.5% of ^{14}C in TG. In this series, an average of 0.52% of the chyle ^{14}C radioactivity was in 20:5 (Table 4B).

DISCUSSION

The study indicates that both villus and crypt cells of the small intestine from rats fed an ordinary pellet diet contain the desaturase (Δ6 and Δ5 desaturase) and elongase systems necessary for the conversion of 18:3 (n-3) to 20:5 (n-3). Although the intracellular location of the enzymes was not studied in detail, microsomal preparations of both villus and crypt cells as well as the 12,000-*g* pellet contained desaturase activity.

The villus cell homogenates catalyzed the interconversion at a higher rate than the crypt cell homogenates. The data thus do not support the idea that fatty acid interconversion in the small intestinal epithelium is most active in the crypts and linked to the need for arachidonic acid as material for membrane phospholipid formation during cell regeneration. Rather the data indicate that the level of desaturase-elongase activity is retained and even increases as the crypt cells differentiate to mature villus cells along the villus-crypt axis. The villus cells will continuously need arachidonic acid and other polyunsaturated fatty acids for formation of the phospholipids of the chylomicron surface coat (2, 3) and for eicosanoid production (15, 16). Our data suggest that desaturation-elongation reactions in the mature villus cells contribute to the need of these cells for 20:4, but the quantitative importance is hard to evaluate at present. This may vary with dietary conditions, as indicated by a recent study showing an up-regulation of the Δ6 desaturase in the intestinal mucosa after fasting for relatively short periods of time (6). Interestingly, Alessandri et al. (17, 18) recently showed that the ratio of 20:4/18:2 was higher in crypt than in villus cells both in piglets and in rats. Our data would thus suggest that the cell fraction with the lowest 20:4/18:2 ratio has the highest Δ6 desaturase activity.

TABLE 4. Incorporation ^{14}C fatty acids into triacylglycerols and phospholipids and interconversion of ^{14}C-radioactive fatty acids to eicosapentaenoic acid in mesenteric duct-cannulated rats

	% Distribution of Radioactivity		
Treatment	TG	PL	% of ^{14}C as 20:5
A. Intralipid			
Jejunum mucosa (n = 3)	88.6 ± 3.1	4.5 ± 1.0	n.d.
Ileum mucosa (n = 3)	86.0 ± 2.9	5.5 ± 1.7	n.d.
Chyle (n = 3)	95.4 ± 1.3	1.3 ± 0.4	n.d.
B. Trilinolenin			
Jejunum mucosa (n = 4)	22.3 ± 4.6	55.6 ± 6.8	n.d.
Ileum mucosa (n = 4)	27.0 ± 2.5	44.3 ± 5.0	n.d.
Chyle (n = 7)	82.5 ± 8.5	3.55 ± 2.4	0.52 ± 0.37

Data under A were obtained from rats which were infused intraduodenally with [^{14}C]18:3 in Intralipid emulsion for 2 h. Chyle was collected from the start of the infusion, and the small intestinal mucosal scrapings were obtained 4 h after the start of infusion, when the rats were killed. Data under B were obtained from rats which were infused [^{14}C]18:3 in an emulsion containing 5 mg trilinolenin. Other experimental conditions are described under Methods. Data are means ± SEM and n.d., not detectable.

To examine the degree of interconversion of dietary [^{14}C]18:3 in the absorptive villus cells under the dietary conditions used, [^{14}C]18:3 was also fed to mesenteric duct-cannulated rats. The interconversion was examined after feeding [^{14}C]18:3 either in a linoleate-rich meal (Intralipid) or with a small amount of trilinolenin. The purpose was to compare the interconversion of 18:3 in the situation where it is the only external substrate and in the situation where dietary 18:3 and a relatively large amount of dietary 18:2 compete for the same desaturating-elongating enzymes. With Intralipid, a linoleate-rich soybean lipid emulsion, as a fat vehicle no measurable amount of ^{14}C appeared in 20:5 in either chyle or mucosal lipids, whereas in the trilinolenin series an average of only 0.68% of the chyle radioactivity was in 20:5. The desaturase-elongase system of the villus cells thus interconvert little or no absorbed dietary 18:3 before incorporation into chylomicrons under the conditions used.

As the action of desaturase-elongase system may be influenced by the rates at which the 18:3-CoA are utilized by other reactions, the degree of interconversion of [^{14}C]18:3 was also related to the rate of acylation into phospholipids and TG. Relatively large amounts of 1-lyso-PC formed from bile phospholipids are continuously absorbed in vivo and are in part reacylated with polyunsaturated fatty acids in the mucosa by the acyl-CoA:1-acyl-GPC acyltransferase (19–22). In our in vitro experiments, addition of 1-palmitoyl-GPC significantly stimulated the incorporation of ^{14}C into PC and decreased the interconversion as well as the incorporation into TG and PA, indicating that the rapid acylation of lyso PC may indeed influence other reactions. In in vivo experiments with trilinolenin infusion, [^{14}C]18:3 was, however, acylated mainly into PL, less proportion being in chyle TG. The efficient acylation into both PC and TG rather than a high rate of lyso PC reacylation may therefore limit the interconversion in vivo.

The acylation patterns in vitro differed between villus and crypt cell homogenates. As a general feature, more [^{14}C]18:3 was incorporated into PC in the presence of 1-acyl-GPC, and less into TG and PA in the crypt than in the villus cells. At moderate concentrations of 1-acyl-GPC the ^{14}C PC formation was also stimulated more strongly in the crypt than in the villus cell homogenates. It is thus concluded that not only the villus but also the crypt cells contain high levels of acyl-CoA:1-acyl-GPC acyltransferase.

1-O-octadecyl-2-O-methyl-*sn*-glycero-3-phosphocholine (PAF-18) is one of several alkyl lyso-phospholipids (ALP) representing a family of new antitumor drugs (23–25). They are closely related structurally to the fatty acid acceptor 1-acyl-GPC. Therefore, it has been suggested that PAF-18, where the 2-position of the glycerol backbone contains a methoxy substituent, is an inhibitor of the acylating enzyme (12, 26, 27). In this study we used PAF-18 to see whether more [^{14}C]18:3 could be channeled to the interconversion reactions by inhibiting the lyso PC acylating enzyme with PAF-18. PAF-18 inhibited, however, not only the acylation of radioactive fatty acids into phospholipids particularly in the crypt cells, but also decreased the interconversion of [^{14}C]18:3 and its acylation into TG. In the intestinal cell homogenates it could thus not be used as a means to change the partitioning of [^{14}C]18:3 between interconversion and acylation.

Our data thus demonstrate the presence of the desaturase-elongase system necessary for formation of 20:5 from 18:3 and thereby also for the formation of 20:4 from 18:2 in both crypt and villus cell of rat small intestine. It is suggested that the high level of acyl-CoA:1-acyl-GPC acyltransferase in the crypt cells serves to favor the retention of polyunsaturated fatty acids in phospholipids during cell regeneration when these cells have to acquire material from blood for the formation of new membrane phospholipids as the cells divide. During the continuous phospholipid remodelling as the cells differentiate into mature villus cells, fatty acid interconversions may occur that contribute to the pools of eicosanoid precursor fatty acids. The quantitative importance of this reaction is likely to be influenced by dietary factors affecting both the spectrum of fatty acids that the crypt cells acquire from blood during cell regeneration and of fatty acids absorbed by the mature villus cells. Although present in the absorptive villus cells, the desaturase-elongase systems interconvert only a minor part of dietary 18:3 (n-3) before its incorporation into chyle lipids.

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REFERENCES

1. Garg, M. L., M. Keelan, A. B. R. Thomson, and M. T. Clandinin. 1990. Intestinal microsomes: polyunsaturated fatty acid metabolism and regulation of enterocyte transport properties. *J. Physiol. Pharmacol.* **68:** 636–641.
2. Brindley, D. N. 1977. Intestinal permeation. *Excerpta Med.* **4:** 350–362.
3. Glickman, R. M. 1984. Lipid adsorption. *In* The Role of the Gastrointestinal Tract in Nutrient Delivery. M. Green and H. L. Greene, editors. Orlando Academic Press, London. 145–156.
4. Borgström, B. 1976. Phospholipid absorption. *In* Lipid Absorption: Biochemical and Clinical Aspects. K. Rommel, H. Goebell, and R. Bohmer, editors. University Park Press, Baltimore. 65–70.
5. Noma, A. 1964. Studies on the phospholipid metabolism of the intestinal mucosa during fat absorption. *J. Biochem. (Tokyo)* **56:** 522–532.

6. Garg, M. L., M. Keelan, A. B. R. Thomson, and M. T. Clandinin. 1992. Desaturation of linoleic acid in the small bowel is increased by short-term fasting and by dietary content of linoleic acid. *Biochim. Biophys. Acta.* **1126:** 17–25.
7. Garg, M. L., M. Keelan, A. B. R. Thomson, and M. T. Clandinin. 1988. Fatty acid desaturation in the intestinal mucosa. *Biochim. Biophys. Acta.* **958:** 139–141.
8. Chen, Q., and Å. Nilsson. 1993. Desaturation and chain elongation of n-3 and n-6 polyunsaturated fatty acids in the human CaCo-2 cell line. *Biochim. Biophys. Acta.* **1166:** 193–201.
9. Weiser, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. *J. Biol. Chem.* **248:** 2536–2541.
10. Bjerknes, M., and H. Cheng. 1981. Methods for the isolation of intact epithelium from the mouse intestine. *Anat. Rec.* **199:** 565–574.
11. Knickelbein, R. G., P. S. Aronson, and J. W. Dobbins. 1988. Membrane distribution of sodium hydrogen and chloride-bicarbonate exchangers in crypt and villus cell membranes from rabbit ileum. *J. Clin. Invest.* **82:** 2158–2163.
12. Herrmann, D. B. J., and H. A. Neumann. 1986. Cytotoxic ether phospholipids. *J. Biol. Chem.* **261:** 7742–7747.
13. Warshaw, A. L. 1972. A simplified method of cannulating the intestinal lymphatic of the rat. *Gut.* **13:** 66–67.
14. Floren, C-H., and Å. Nilsson. 1977. Binding, interiorization and degradation of cholesteryl ester-labeled chylomicron remnant particles by rat hepatocyte monolayers. *Biochem. J.* **168:** 483–494.
15. Knapp, H. R., O. Olez, B. J. Sweetman, and J. A. Oates. 1978. Synthesis and metabolism of prostaglandins E2, F2 and D2 by the rat gastrointestinal tract: stimulation by a hypertonic environment in vitro. *Prostaglandins.* **15:** 751–757.
16. LeDuc, L. E., and P. Needleman. 1979. Regional localization of prostacyclin and thromboxane synthesis dog stomach and intestinal tract. *J. Pharmacol. Exp. Ther.* **211:** 181–188.
17. Alessandri, J. M., P. Guesnet, T. S. Arfi, and G. Durand. 1991. Changes in fatty acid composition during cell differentiation in the small intestine of suckling piglets. *Biochim. Biophys. Acta.* **1086:** 340–348.
18. Alessandri, J. M., J. L. Joannic, and G. Durand. 1993. Polyunsaturated fatty acids as differentiation markers of rat jejunal epithelial cells: a modeling approach. *J. Nutr. Biochem.* **4:** 97–104.
19. Mansbach, J. C. M. 1977. The origin of chylomicron phosphatidylcholine in the rat. *J. Clin. Invest.* **60:** 411–420.
20. Nilsson, Å. 1968. Intestinal absorption of lecithin and lysolecithin by lymph fistula rats. *Biochim. Biophys. Acta.* **152:** 379–390.
21. Scow, R. O., Y. Stein, and O. Stein. 1967. Incorporation of dietary lecithin and lysolecithin into lymph chylomicrons in the rat. *J. Biol. Chem.* **242:** 4919–4924.
22. Tso, P., J. A. Balint, and W. J. Simmonds. 1977. Role of biliary lecithin in lymphatic transport of fat. *Gastroenterology.* **73:** 1362–1367.
23. Berdel, W. E., W. R. Bausert, H. U. Weltzien, M. L. Modolell, K. H. Widmann, and P. G. Munder. 1980. The influence of alkyl-lysophospolipids and lysophospholipid-activated macrophages on the development of metastasis of 3 Lewis lung carcinoma. *Eur. J. Cancer.* **16:** 1199–1204.
24. Berdel, W. E., H. Schlehe, U. Fink, B. Emmerich, P. A. Maubach, H. P. Emslander, S. Daum, and J. Rastetter. 1982. Early tumor and leukemia response to alkyl-lysophospholipids in a phase I study. *Cancer.* **50:** 2011–2015.
25. Munder, P. G., M. Modolell, W. Bausert, H. F. Oettgen, and O. Westphal. 1981. Alkyl-lysophospholipids in cancer therapy. *In* Augmenting Agents in Cancer Therapy. E. M. Hersh, M. A. Chirigos, and M. J. Mastrangelo, editors. Raven Press, New York. 441–458.
26. Storme, G. A., W. E. Berdel, W. J. van Blitterswijk, E. A. Bruyneel, G. K. De Bruyne, and M. M. Mareel. 1985. Antiinvastive effect of racemic 1-O-octadecyl-2-O-methylglycero-3-phosphocholine on MO_4 mouse fibrosarcoma cell in vitro. *Cancer Res.* **45:** 351–357.
27. Andreesen, R., M. Modolell, H. U. Weltzien, H. Eibl, H. H. Common, G. W. Löhr, and P. G. Munder. 1978. Selective destruction of human leukemic cells by alkyl-lysophospholipids. *Cancer Res.* **38:** 3894–3899.