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# Localization and capacity of sphingomyelin digestion in the rat intestinal tract

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*Dietary sphingomyelin (SM) undergoes sequential cleavage to ceramide and sphingosine in the intestine. A distinctive intestinal sphingomyelinase (SMase) with alkaline pH-optimum was earlier identified by us. The activity was highest in middle and lower small intestine, but its role in SM digestion has not been clarified. In this study we examined the extension and capacity of SM digestion in vivo. After feeding rats 0.2, 6.6, or 32 μmol SM containing 2 μCi $^3$H-sphingosine-labeled milk SM ($^3$H-SM), radioactivity was analyzed in intestinal contents and tissues 2, 4, and 8 hr later. The proportion of radioactivity in the contents of small intestine increased with the dose of SM; 9% of given dose with 0.2 μmol, 34% with 6.6 μmol, and 71% with 32 μmol, respectively after 2 hr. Lowest tissue radioactivity was found in duodenum and proximal jejunum and highest in distal jejunum and proximal ileum. Three to twenty one percent of radioactivity in the intestinal tissue was in ceramide, the proportion varying with the dose given, region of the intestine, and time after administration. After administration of 6.6 or 32 μmol SM, significant amounts of intact SM and ceramide was found in intestinal contents, colon, and excreted faeces. Colon was exposed to ceramide derived from exogenous SM in amounts that were rather proportional to the dose of SM fed. SM digestion is thus a process extending over the whole intestine and occurring mainly in the middle and lower parts of the small intestine. The site of digestion coincides with the distribution of the alkaline SMase, indicating that this enzyme catalyzes the first step in the digestion. The extension and limited capacity of the SM digestion leads to an exposure of the lower small intestine and colon to SM and sphingolipid metabolites.* (J. Nutr. Biochem. 8:112–118, 1997.) © *Elsevier Science Inc. 1997*

**Keywords:** sphingomyelin digestion; ceramide formation; intestine; rat

## Introduction

Sphingomyelin (SM) is a dietary component that occurs mainly in milk, egg, meat, and fish.[1–4] Sphingolipid derivatives such as ceramide and sphingosine have been shown to be important lipid messengers that regulate cell proliferation, differentiation, and apoptosis.[5–7] The potential biologic effects of dietary and endogenous sphingolipid metabolites on the gastrointestinal tract has, therefore, become an interesting research area. Whereas dietary triacylglycerol- and glycerophospholipids are rapidly hydrolyzed by pancreatic enzymes in the upper small intestine, no digestive enzyme in pancreatic juice that hydrolyzes SM has been demonstrated. A sphingomyelinase (SMase) activity with alkaline pH optimum was first identified by Nilsson in human and pig intestinal contents and rat intestinal brush border.[8,9] Recently, we further characterized the intestinal alkaline SMase in rat intestinal mucosa and contents.[10] The level of alkaline SMase was low in the duodenum and reached its highest level in the middle of the small intestine. The enzyme was also present in colon and rectum. This distribution pattern differs distinctly from those of alkaline phosphatase, acid SMase, and other brush border enzymes as disacharidases and peptidases. Furthermore, a novel alkaline SMase activity was recently identified in human

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bile by Nyberg et al.[11] These alkaline SMases could play important roles in digestion of dietary SM.

Although the role of individual enzymes has not been established, early studies indicated that most of the dietary SM undergoes sequential cleavage to ceramide and phosphocholine, followed by hydrolysis of the ceramide to sphingosine and fatty acid, and oxidation in the mucosal cells of sphingosine to palmitic acid.[8,9] Recently, Schmelz et al.[12] confirmed and extended these observations in experiments using an isolated intestinal loop model in mice, and suggested that the ability to degrade luminal SM is a feature of most parts of the gastrointestinal tract.

Many questions about SM digestion and absorption remain, however, elusive. For instance it has not been established whether the location of SM digestion is in accordance with the distribution of the alkaline SMase in the intestine. The capacity of the gut to digest SM and the influence of different amounts of dietary SM on intracellular and extracellular ceramide have not been characterized. In the present work, we examined the digestion of SM and thus ceramide formation at different levels of the rat intestinal tract after administration of different amounts of SM. Our results support an important role of alkaline SMase in SM digestion and show that the amount of dietary SM influences ceramide levels in the intestinal tract.

## Methods and Materials

### *Materials*

Male white Sprague-Dawley rats, weighing approximately 200 g, were obtained from Møllegaard, Denmark, housed in a temperature-controlled room under a 12-hr light and dark rhythm, and fed a commercial standard pellet diet containing 18% protein, 4% fat, and 13% carbohydrate, supplemented with vitamins and minerals (Altromin, Denmark), with free access to water. SM was provided from The Swedish Dairies' Association, which has developed a patented technique for isolating SM, using butter milk or whey as raw materials.[13] The purity of SM, as analyzed on HPLC,[14] was >95%. The composition of SM from bovine milk is quite complex,[15] with the dominating acyl groups being C16:0 (34.4%), C23:0 (21.4%), C22:0 (17.0%), C24:0 (13.5%), and sphingoid bases C18:1 (64%), and C16:1 (23%). Sphingosine is used in this work to designate all sphingoid bases. Ceramide, sphingosine, phosphatidylcholine, phosphatidylethanolamine, and palmitic acid, used as standard substances in TLC analysis were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

### *Radiolabeling of SM*

$^{3}$H-sphingosine-labeled milk-SM ($^{3}$H-SM) and (N-$^{14}CH_3$) choline labeled milk-SM ($^{14}$C-SM) were kindly prepared by Peter Ström, Astra Draco, Lund. $^{3}$H-SM was prepared by reducing the double bond in the sphingosine of milk-SM with tritium (Tritec AG) in PdO/methanol. The $^{3}$H labeled SM had a specific activity of 48.5 mCi/mg. $^{14}$C-SM was prepared by a method developed by Stoffel.[16] Briefly, milk-SM was demethylated to ceramide-1-phosphoryl-N,N-dimethylethanolamine with 1,4-diazabicyclo(2,2,2)octane (DABCO) in dimethyl-formamide, followed by methylation with addition of cyclohexylamine and $^{14}CH_3I$. $^{14}$C-SM with a specific activity of 56 μCi/mg was obtained. The purity and identity of the products were determined by $^{1}$H- and $^{13}$C-NMR, TLC, and mass spectrometry. Radiochemical purity for SM was greater than 98%, as estimated by TLC on a plate of silica gel developed in $CHCl_3$ : $CH_3OH$ : $NH_3$ (65:35:8, vol/vol/vol). The radioactivity on the plates was located using a RITA92 TLC-scanner (Raytest, Straubenhardt, Germany).

### *Preparation of SM dispersions*

$^{3}$H-SM or $^{14}$C-SM dissolved in ethanol was transferred to glass tubes and taken to dryness with nitrogen. Unlabeled milk-SM and 6 mL 0.9% sodium chloride solution were added to the glass tubes and the dispersions sonicated for 3 × 1 min. Three mL coffee cream (homogenized cream with a fat content of 12%, w/w) was added to the SM dispersions to a final fat content of 4% (v/v) and mixed on an Ultra Turrax for 15 sec. Dispersions with 0, 3.3, and 16.7 mg unlabeled milk-SM added per mL, were prepared. On calculation with a mean molecular weight of 775 for milk-SM, the SM concentrations of the dispersions were 0.13, 4.4, and 21 μmol per mL, respectively. In the dispersion with lowest SM concentration (0.13 μmol/mL), the SM mass originates from added cream.

### *Animal experiments*

After fasting for 24 hr with free access to tap water, rats were fed 1.5 mL dispersion containing different amounts of SM, as mentioned previously, by gastrogavage. In the first series, rats were fed 0.2, 6.6, or 32 μmol unlabeled milk-SM and 2μCi $^{3}$H-SM (specific radioactivity in the three different SM-doses: 10 mCi $^{3}$H/mmol SM, 0.3 mCi $^{3}$H/mmol SM, and 0.06 mCi $^{3}$H/mmol SM, respectively). After 2, 4, and 8 hr, the rats were anaesthetized with diethyl ether and killed by puncturing the aorta. Stomach, small intestine, colon, and liver were removed. The small intestine was divided distally in four equal parts designated as level 1, 2, 3, and 4, which represent the duodenum and proximal jejunum, the middle jejunum, the distal jejunum and proximal ileum, and the ileum, respectively. Each part was rinsed with 0.9% sodium chloride solution, and intestinal contents in washing fluids were collected. Lipids in both intestinal contents and tissues were extracted as described below. In a second experimental series, additional rats were fed 0.2 or 6.6 μmol unlabeled milk-SM and 1 μCi choline-labeled $^{14}$C-SM (specific radioactivity in the two different SM-doses: 5 mCi $^{14}$C/mmol SM and 0.15 mCi $^{14}$C/mmol SM, respectively). The rats were killed 2 or 4 hr thereafter and the livers were removed. In a third series of experiments, non-fasted rats were fed 1.5 mL of SM dispersions containing 0.2, 6.6, or 32 μmol unlabeled milk-SM and 2 μCi $^{3}$H-SM and the feces thereafter collected for 24 hr.

### *Lipid analysis*

Lipids were extracted from the intestinal contents, tissues, and feces, with chloroform : methanol : water according to Bligh and Dyer.[17] Aliquots of the chloroform and aqueous

phases were taken for liquid scintillation counting. On plates of silica gel (Merck, Si 60-F, 0.25 mm) aliquots of the chloroform phase were taken for TLC analysis. In experiments with $^{3}$H-sphingosine labeled SM the plates were developed in chloroform : methanol : ammonia (100:15:1.5, v/v/v), which separates SM and its hydrolysis products ceramide, sphingosine, and triglycerides (fatty acids). In this solvent system SM and other phospholipids stay at the start, whereas triglycerides migrate with the front. Rf-values for sphingosine and ceramide were 0.3 and 0.7, respectively. Free fatty acids with Rf-value 0.1 are included in polar lipids in the presentation of results. To separate polar lipids, the silica gel plates were developed in chloroform : methanol : water : acetic acid (65:35:4:4, v/v/v). The distribution of $^{3}$H between SM, ceramide, sphingosine, major glycerophospholipids, and total nonpolar lipids was thus examined, but not the incorporation into more complex glycosphingolipids. The spots were identified by the use of standard substances, visualized with iodine, and scraped into scintillation vials. Radioactivity of total lipid extracts and of thin layer scrapings was determined by liquid scintillation counting.

## Results

### *Radioactivity in different tissues*

When rats were fed three different amounts of SM (0.2, 6.6, and 32 μmol respectively), the duodenal and proximal jejunal tissues (level 1) contained low radioactivity with all three doses (*Figure 1*). Highest activity was found in the middle and lower parts of the small intestine (level 2–4), indicating that the digestion and absorption of SM were most active in these intestinal segments. In rats fed the lowest SM dose (0.2 μmol), most of the radioactivity was found in the tissues of the middle part of the intestine. Radioactivity in ileum was found mainly in the contents. With increased SM dose, the major part of radioactivity was found in the contents, particularly in the distal small intestine (level 3 and 4). Two hr after feeding 6.6 μmol SM, radioactivity in the contents of level 3 and 4 was 32% of the given dose, and increased to 69% when rats were fed 32 μmol SM. Eight hr after administration of SM, radioactivity in the intestinal contents had decreased to a level that was lower than that in the tissues.

The recovery of radioactivity in stomach, whole small intestine (tissue and content analyzed separately), colon, and liver is shown in *Table 1*. A higher share of given radioactivity remained in the stomach 4 hr after feeding rats the lowest SM dose, 13% compared to 3.5% with the higher doses. More than 95% of the radioactivity was found as SM, indicating that little hydrolysis of SM occurred in the stomach. Significant radioactivity in colon was found only after 8 hr, amounting to 2 to 4% of the given dose. Recovery of radioactivity in the liver with $^{3}$H-sphingosine labeled SM was 1 to 4%, consisting of about equal parts of polar lipids, ceramide, and nonpolar lipids. With $^{14}$C-choline labeled SM, radioactivity in liver was significantly higher, 26 to 36% (*Table 1*). Two-thirds of the $^{14}$C-radioactivity in liver was chloroform soluble, and mainly as phosphatidylcholine (>95%), confirming earlier data[8,9] that absorption and

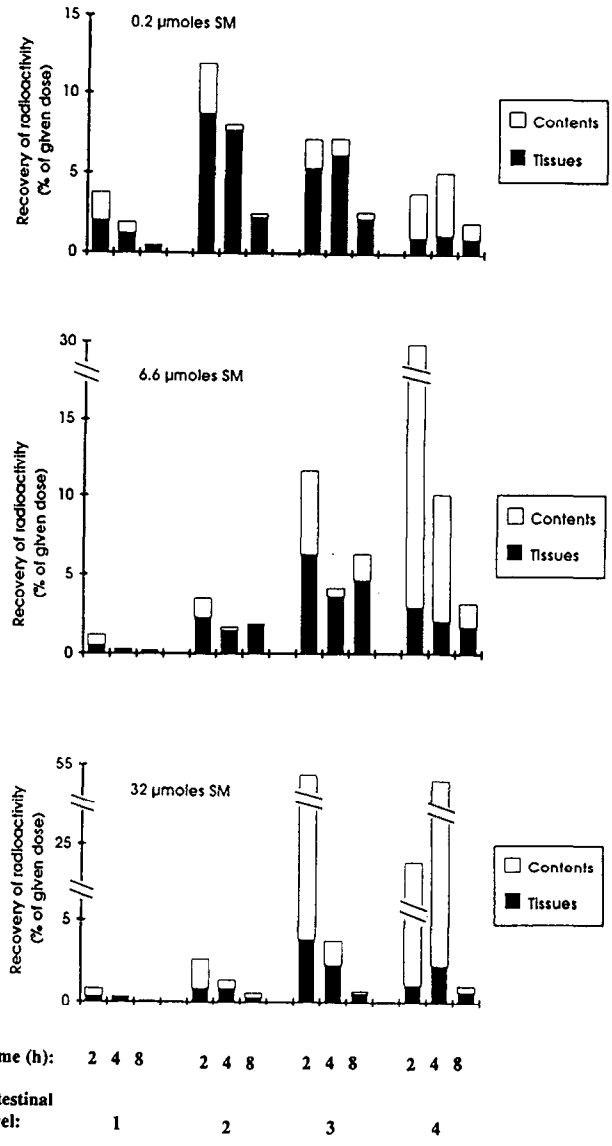


**Figure 1** Recovery of radioactivity in the small intestine. Rats were fed 0.2, 6.6, or 32 μmol milk-SM, containing 2 μCi $^{3}$H-SM and the course of digestion and uptake along the intestine was followed by analysis of recovered radioactivity in different parts of the gastrointestinal tract 2, 4, and 8 hr after administration. The small intestine was equally divided into four segments, termed level 1, 2, 3, and 4, which represent duodenum and proximal jejunum, middle jejunum, the distal jejunum and proximal ileum, and the distal ileum, respectively. Lipid radioactivity in intestinal contents and tissues from each segment was analyzed separately. Results are means from three rats. The standard errors, in most cases, are less than 30% of the means.

transport to the liver of intact dietary SM was negligible. Less than 0.1% of radioactivity was found in blood plasma with all three doses and at all time intervals examined.

### *Identification and quantification of degradation products in the intestine*

To identify in which form radioactivity existed in different parts of the gastrointestinal tract, aliquots of lipid extracts from tissues and contents were analyzed by TLC. The

**Table 1** Recovered radioactivity in stomach, whole small intestine, colon and liver (% of given radioactive SM dose)

| | | Small Intestine | | | Liver | |
|---|---|---|---|---|---|---|
| SM | Stomach $^{3}$H-SM | Tissues $^{3}$H-SM | Contents $^{3}$H-SM | Colon $^{3}$H-SM | $^{3}$H-SM | $^{14}$C-SM |
| 0.2 μmol | | | | | | |
| 2 hr | 13.5 ± 3.8 | 17.2 ± 2.1 | 9.4 ± 2.3 | 0.1 ± 0.1 | 2.5 ± 0.3 | 26.2 ± 7.2 |
| 4 hr | 13.1 ± 4.5 | 16.3 ± 2.8 | 5.9 ± 1.3 | 0.3 ± 0.2 | 4.1 ± 0.9 | 29.3 ± 6.8 |
| 8 hr | 0.8 ± 0.4 | 6.0 ± 0.8 | 1.6 ± 0.3 | 2.0 ± 1.5 | 3.8 ± 0.3 | nd |
| 6.6 μmol | | | | | | |
| 2 hr | 6.8 ± 1.6 | 12.0 ± 0.1 | 34.2 ± 2.4 | 0.1 ± 0.0 | 1.6 ± 0.2 | 27.8 ± 2.2 |
| 4 hr | 3.5 ± 1.8 | 7.4 ± 1.8 | 8.8 ± 3.4 | 0.7 ± 0.6 | 2.0 ± 0.5 | 36.2 ± 2.7 |
| 8 hr | 2.7 ± 1.3 | 8.4 ± 0.4 | 3.2 ± 0.6 | 1.7 ± 0.8 | 3.6 ± 0.4 | nd |
| 32 μmol | | | | | | |
| 2 hr | 10.7 ± 2.5 | 6.1 ± 0.6 | 71.7 ± 1.0 | 0.03 ± 0.0 | 0.4 ± 0.0 | nd |
| 4 hr | 3.6 ± 0.1 | 5.7 ± 1.1 | 51.1 ± 3.7 | 0.04 ± 0.0 | 0.8 ± 0.1 | nd |
| 8 hr | 0.1 ± 0.0 | 1.7 ± 0.2 | 0.7 ± 0.3 | 4.0 ± 3.3 | 0.8 ± 0.1 | nd |

Radioactivity in stomach, intestine (tissue/content), colon and liver, respectively, 2, 4, and 8 hr after feeding rats different amounts of SM containing $^{3}$H milk-SM and/or $^{14}$C milk-SM ($^{3}$H SM labeled in the sphingoid base and $^{14}$C SM choline labeled). For details, see Methods. The results are expressed as percent of given dose, mean value ± SEM from three rats.
nd, not determined.

results from level 2 to 4 are shown in *Table 2*. Radioactivity was found in polar and neutral lipids, sphingoid bases, and ceramide in all extracts from both intestinal contents and tissues. The percentage of lipid radioactivity as ceramide in the intestinal tissue ranged from 3 to 21%, depending on SM-dose, time after feeding and level of the small intestine. The distal part of small intestine seemed to contain a higher proportion of radioactive ceramide than the proximal level. A few percent of radioactivity was found in free sphingosine in the whole intestine. The neutral lipids accounted for a higher share of total radioactivity in the intestinal tissues than in the contents (*Table 2*), indicating that ceramidase activity and further degradation of sphingosine take place in the brush border of the intestinal wall. The major part of radioactivity in both tissues and contents consisted of polar lipids (including fatty acids), being 46 to 87% in the tissues and 61 to 97% in the contents. The share of polar lipids was increasing with time in the tissues and unchanged or decreasing in the contents. The SM part of polar lipids was analysed and the results from level 3 is shown in *Table 3*, where SM made up 45 to 84% of polar lipids, decreasing with time. In the colon the ceramide part of recovered radioactivity was 20 to 32% (*Table 4*), the amount of ceramide thus increasing with the dose of SM given to the animals.

### *Feces*

Recovered radioactivity in feces, collected for 24 hr, is given in *Table 5*. With the lowest SM dose (0.2 μmol), 32% of the given radioactivity was recovered in feces. The recovery increased to 45% with the highest dose (32 μmol). 49 to 73% of the radioactivity in feces was in polar lipids (including fatty acids), of which 90% was SM. With all three doses, about 20% of recovered radioactivity in feces was present as ceramide. The mass amount of exogenous ceramide in feces thus increased proportionally with SM intake (*Figure 2*).

## Discussion

This study confirms that after oral administration, SM is hydrolyzed to ceramide, which is further degraded to free sphingosine and fatty acid. In this respect, our data confirm earlier studies.[9,12] Enzymes that may catalyze this sequence of reactions were identified previously, but their role in SM digestion has not been clearly established. Nilsson[8] demonstrated a bile salt dependent SMase in the mucosa of the small intestine and in small intestinal contents, which had an alkaline pH-optimum and which was enriched in brush border preparations. A ceramidase activity with a neutral pH-optimum was also found in intestinal mucosa and contents.[8] Our results in the present study support a role of the alkaline SMase in the digestion of SM. The incorporation of radioactivity in the intestinal tissues after $^{3}$H-SM administration was highest in the middle of jejunum to the proximal ileum. The intestinal contents of these segments also contained more ceramide than the proximal parts of the small intestine, in which mainly undigested SM was found. Recently, we reported that the alkaline SMase was almost absent in the duodenum, increased in proximal jejunum, and reached the highest levels in the distal jejunum and proximal ileum.[10] The conclusion is thus that the digestion of SM occurs more distally than the digestion of glycerolipids, and mainly in the parts of the small intestine where alkaline SMase is most abundant. The possibility that dietary SM is hydrolyzed by the acid SMase shown to be present in small intestine, but most abundant in duodenum,[10] seems unlikely. Neither is alkaline phosphatase likely to be identical to alkaline SMase or to have a role in SM digestion, because the levels were highest in the duodenum[10] and the purified enzyme was shown to be inactive against SM.[8]

Our study also shows that the hydrolysis and absorption of SM in the intestine is slow and incomplete, with a relatively low capacity. Because the given SM is mixed with endogenous sphingolipids from sloughed mucosal cells, this could perhaps explain that also with the lowest dose of SM

**Table 2** Percent distribution of polar lipids, neutral lipids, ceramide and sphingosine in intestinal lipid extracts

| | Polar Lipids | | Sphingosine | | Ceramide | | Neutral Lipids | |
|---|---|---|---|---|---|---|---|---|
| | Tissues | Contents | Tissues | Contents | Tissues | Contents | Tissues | Contents |
| | | | | Small Intestine, level 2 | | | | |
| 0.2 μmol | | | | | | | | |
| 2 hr | 62 ± 7 | 82 ± 4 | 3 ± 3 | 0 ± 0 | 11 ± 3 | 12 ± 3 | 25 ± 5 | 6 ± 3 |
| 4 hr | 79 ± 1 | 91 ± 4 | 2 ± 2 | 0 ± 0 | 5 ± 1 | 5 ± 5 | 14 ± 4 | 4 ± 1 |
| 8 hr | 86 ± 2 | 79 ± 6 | 1 ± 0 | 1 ± 1 | 4 ± 1 | 3 ± 1 | 9 ± 1 | 17 ± 5 |
| 6.6 μmol | | | | | | | | |
| 2 hr | 65 ± 9 | 93 ± 4 | 0 ± 1 | 0 ± 0 | 8 ± 2 | 6 ± 3 | 27 ± 8 | 1 ± 0 |
| 4 hr | 84 ± 6 | 94 ± 3 | 1 ± 1 | 0 ± 0 | 3 ± 1 | 5 ± 3 | 11 ± 5 | 0 ± 0 |
| 8 hr | 82 ± 2 | 79 ± 4 | 1 ± 0 | 1 ± 1 | 4 ± 1 | 4 ± 1 | 13 ± 2 | 15 ± 3 |
| 32 μmol | | | | | | | | |
| 2 hr | 66 ± 7 | 97 ± 1 | 1 ± 0 | 1 ± 0 | 6 ± 1 | 2 ± 1 | 27 ± 6 | 1 ± 0 |
| 4 hr | 69 ± 4 | 91 ± 0 | 1 ± 0 | 2 ± 1 | 5 ± 1 | 5 ± 1 | 25 ± 4 | 3 ± 2 |
| 8 hr | 74 ± 3 | 76 ± 23 | 3 ± 0 | 1 ± 1 | 6 ± 1 | 1 ± 1 | 18 ± 4 | 22 ± 2 |
| | | | | Small Intestine, level 3 | | | | |
| 0.2 μmol SM | | | | | | | | |
| 2 hr | 53 ± 4 | 61 ± 4 | 4 ± 4 | 4 ± 1 | 9 ± 4 | 20 ± 6 | 33 ± 2 | 15 ± 3 |
| 4 hr | 75 ± 3 | 73 ± 1 | 3 ± 3 | 3 ± 2 | 5 ± 1 | 11 ± 2 | 17 ± 3 | 13 ± 4 |
| 8 hr | 82 ± 1 | 85 ± 4 | 1 ± 0 | 4 ± 2 | 5 ± 0 | 6 ± 2 | 12 ± 1 | 6 ± 1 |
| 6.6 μmol SM | | | | | | | | |
| 2 hr | 53 ± 1 | 81 ± 2 | 1 ± 1 | 1 ± 1 | 10 ± 2 | 12 ± 4 | 38 ± 4 | 3 ± 1 |
| 4 hr | 74 ± 3 | 77 ± 6 | 2 ± 2 | 1 ± 0 | 6 ± 2 | 13 ± 1 | 18 ± 3 | 11 ± 1 |
| 8 hr | 64 ± 2 | 72 ± 4 | 0 ± 0 | 12 ± 2 | 6 ± 1 | 13 ± 1 | 29 ± 2 | 3 ± 1 |
| 32 μmol SM | | | | | | | | |
| 2 hr | 72 ± 6 | 96 ± 0 | 6 ± 1 | 0 ± 0 | 5 ± 1 | 2 ± 0 | 23 ± 6 | 1 ± 0 |
| 4 hr | 46 ± 8 | 87 ± 6 | 12 ± 6 | 4 ± 2 | 10 ± 3 | 8 ± 4 | 33 ± 7 | 1 ± 1 |
| 8 hr | 72 ± 10 | 83 ± 14 | 6 ± 3 | 0 ± 0 | 10 ± 5 | 2 ± 1 | 12 ± 2 | 14 ± 13 |
| | | | | Small Intestine, level 4 | | | | |
| 0.2 μmol | | | | | | | | |
| 2 hr | 55 ± 3 | 64 ± 4 | 4 ± 4 | 3 ± 1 | 17 ± 1 | 26 ± 6 | 23 ± 4 | 7 ± 2 |
| 4 hr | 76 ± 5 | 70 ± 5 | 2 ± 1 | 1 ± 1 | 10 ± 3 | 21 ± 6 | 12 ± 2 | 7 ± 1 |
| 8 hr | 80 ± 2 | 75 ± 2 | 2 ± 1 | 5 ± 1 | 4 ± 1 | 11 ± 2 | 14 ± 3 | 9 ± 2 |
| 6.6 μmol | | | | | | | | |
| 2 hr | 62 ± 4 | 71 ± 3 | 4 ± 2 | 0 ± 0 | 21 ± 4 | 21 ± 6 | 13 ± 1 | 1 ± 0 |
| 4 hr | 69 ± 4 | 72 ± 5 | 4 ± 3 | 1 ± 0 | 13 ± 3 | 23 ± 6 | 13 ± 2 | 4 ± 1 |
| 8 hr | 80 ± 1 | 81 ± 4 | 0 ± 0 | 1 ± 0 | 4 ± 0 | 10 ± 3 | 16 ± 1 | 8 ± 1 |
| 32 μmol | | | | | | | | |
| 2 hr | 73 ± 12 | 87 ± 6 | 0 ± 0 | 2 ± 1 | 5 ± 1 | 10 ± 3 | 20 ± 11 | 2 ± 2 |
| 4 hr | 72 ± 6 | 88 ± 1 | 5 ± 1 | 1 ± 0 | 9 ± 0 | 10 ± 1 | 14 ± 5 | 1 ± 0 |
| 8 hr | 87 ± 2 | 55 ± 16 | 0 ± 0 | 10 ± 7 | 4 ± 0 | 28 ± 21 | 9 ± 2 | 8 ± 5 |

Percent distribution of polar lipids, neutral lipids, ceramide, and sphingosine in intestinal segments 2, 4, and 8 hr after feeding rats three different amounts of unlabeled milk-SM with 2 μCi $^{3}$H-SM. The distribution in contents and tissues are calculated separately for each segment. Fatty acids are included in polar lipids. Results are expressed as mean ± SEM from three rats.

(0.2 μmol), radioactive SM and ceramide were found in feces. The doses 6.6 and 32 μmol SM may be beyond the capacity of rat intestine to metabolize, because radioactivity in SM and in ceramide in the contents of lower small intestine was high with these doses. Another interesting finding is that when the dose of SM fed increased by 160 times, the percentage recovery of radioactivity in feces was increased only slightly (*Table 5*). This result, which is in good agreement with previous findings,[9] indicate that the intestine, to some extent, only takes up certain percentage of SM, which is less dependent on the dose of SM fed. The mechanism for this regulation is unknown.

**Table 3** Percent sphingomyelin of phospholipids in small intestinal tissue, at level 3

| | Sphingomyelin dose (μmol) | | |
|---|---|---|---|
| Time (hr) | 0.2 | 6.6 | 32 |
| 2 | 71 ± 3 | 58 ± 5 | 84 ± 3 |
| 4 | 73 ± 2 | 54 ± 6 | 63 ± 8 |
| 8 | 47 ± 3 | 46 ± 2 | 45 ± 2 |

Percentage SM of total phospholipids in tissues at level 3 of the small intestine 2, 4, and 8 hr, respectively, after feeding rats three different amounts of milk-SM with 2 μCi $^{3}$H-SM. Results are mean ± SEM from three rats.

At present, we do not know which factors are rate-limiting for the digestion of SM and ceramide. The SMase may act under suboptimal conditions because of its high optimal pH, and physical factors may limit the exposure of the substrates to the SMase and ceramidase activities. Other luminal factors may also be important, e.g. dihydroxy bile salts was found to inhibit alkaline SMase activity (Rui-Dong Duan et al., unpublished results).

The role of different enzymes in the digestion of ceramide is unknown. Recently, we demonstrated (Nyberg et

**Table 4** Percent distribution of polar lipids, neutral lipids, ceramide and sphingosine in colon

| | Polar Lipids | Sphingosine | Ceramide | Neutral Lipids |
|---|---|---|---|---|
| 0.2 μmol SM 8 hr | 47 ± 7 | 2 ± 1 | 20 ± 9 | 31 ± 6 |
| 6.6 μmol SM 8 hr | 45 ± 9 | 1 ± 1 | 32 ± 2 | 22 ± 8 |
| 32 μmol SM 8 hr | 71 ± 4 | 1 ± 1 | 23 ± 3 | 4 ± 1 |

Distribution of polar lipids, neutral lipids, ceramide, and sphingosine was assayed in colon 8 hr after feeding rats three different amounts of unlabeled milk-SM with 2 μCi $^3$H-SM. The distribution is expressed as percent of total radioactivity in each extract. Fatty acids are included in polar lipids. Results are mean ± SEM from three rats. For details, see Methods.

al., unpublished results) that pancreatic bile salt dependent lipase has ceramidase activity. This enzyme, which can bind to the microvillar membrane of the mucosal cells[18] may actually be identical to the mucosal ceramidase identified in older studies.[8] If so, the function of the enzyme in ceramide digestion may be limited by the fact that the levels are lower in the middle and distal small intestine, where ceramide is actually formed from dietary SM, than in the proximal part. Further studies on the identity, properties, and longitudinal distribution of mucosal and luminal ceramidases are necessary. Teleologically, the absence of a pancreatic SMase and the extended course of SM digestion may prevent the endogenous SM in the microvillar membrane from extensive digestion, which may be crucial to mucosal cell integrity, and yet make digestion of most exogenous SM possible.

Recently, Dillehay et al.[19,20] demonstrated that addition of SM to a normal diet in mice reduced the promotion of colon cancers induced by 1,2-dimethylhydrazine. Although the inhibitory factor has not been established, ceramide may be responsible, because ceramide has been shown to have antiproliferative effects on cell growth and to induce apoptosis.[7,21] It is thus of interest to know whether oral

**Table 5** Lipid radioactivity in feces

| | Sphingomyelin Dose (μmol) | | |
|---|---|---|---|
| | 0.2 | 6.6 | 32 |
| Recovery (% of given dose) | 33 ± 4 | 42 ± 6 | 45 ± 0.3 |
| Lipids (% of recovered radioactivity) | | | |
| Neutral lipids | 24 ± 2 | 19 ± 6 | 7 ± 0 |
| Ceramide | 22 ± 2 | 20 ± 4 | 17 ± 2 |
| Sphingosine | 5 ± 1 | 5 ± 1 | 4 ± 1 |
| Polar lipids | 49 ± 1 | 56 ± 6 | 73 ± 2 |
| Sphingomyelin Phospholipids (%) | 87 ± 0 | 94 ± 1 | 85 ± 8 |

Recovered total radioactivity and percent distribution of polar lipids, neutral lipids, ceramide, and sphingosine in feces 24 hr after feeding rats three different amounts of unlabeled milk-SM with 2μCi $^3$H-SM. Recovered radioactivity is expressed as percent of given dose. The distribution of lipid classes is expressed as percent of radioactivity in each extract. Fatty acids are included in polar lipids. The proportion of sphingomyelin is expressed as percentage of phospholipids. Results are mean ± SEM from three rats.

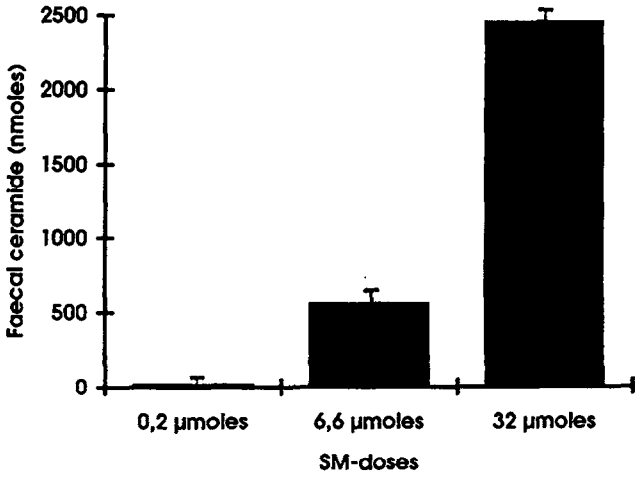


**Figure 2** Ceramide levels in feces. Rats were fed 0.2, 6.6, and 32 μmol milk-SM, containing 2μCi $^3$H-SM and the feces were collected for 24 hr. The lipids in the feces were extracted and the ceramide part isolated by TLC. The amount of ceramide excreted with the feces within 24 hr was calculated. Results are mean ± SEM from three rats.

administration of SM influences the intracellular and extracellular levels of ceramide in the intestinal mucosa, particularly the colonic mucosa. Our experiments suggest that the levels of ceramide in the intestinal tissue and lumen may indeed be influenced by the amount of SM administered, as the amount of ceramide detected in colon and feces was rather proportional to the amount of SM fed. Undigested SM that is transported to the colon can also be degraded locally to ceramide, because the alkaline SMase was present in colonic mucosa.[10] Further studies on the effects of dietary SM on intracellular sphingolipid metabolites at different levels of the gastrointestinal tract are however necessary, to more intrically evaluate the hypothesis that ceramide derived from dietary SM may suppress development of colon cancer.

In summary, our study supports a concept that SM digestion is a process that is extended over the whole small intestine, occurring mainly in the middle and lower parts. The site of digestion coincides with the distribution of the alkaline SMase, indicating that this enzyme catalyzes the first step in the digestion. The extension and the limited capacity of the SM digestion leads to a long-term exposure of the lower small intestine and colon to SM and sphingolipid metabolites.

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