Distribution of molecular species of sphingomyelins in different parts of bovine digestive tract

M. E. Breimer

Membrane Biochemistry Group, Department of Medical Biochemistry, University of Göteborg, Fack, S-400 33 Göteborg 33, Sweden

Abstract Sphingomyelins were isolated from mucosal layers of bovine rennet stomach, duodenum, jejunoileum, and colon ascendens. The ceramides obtained after phospholipase degradation were characterized by thin-layer chromatography, mass spectrometry, and gas–liquid chromatography. The main ceramide group from all regions consisted of dihydroxy long-chain bases and normal fatty acids. Sphingosine was the predominant base in all these fractions, and only in rennet stomach were smaller amounts of the C_{17} and C_{20} homologs present. Normal saturated C_{16}, C_{18}, C_{22}, and C_{24} fatty acids were most abundant. In rennet stomach there was in addition a ceramide group having dihydroxy long-chain bases in combination with hydroxy fatty acids. Sphingosine was the predominant long-chain base and the fatty acids were 2-hydroxy C_{16}, C_{22}, C_{23}, and C_{24}. From jejunoileum three minor ceramide fractions were isolated; these consisted of phytosphingosine and normal fatty acids (C_{22}–C_{24}), sphingosine and 2-hydroxy fatty acids (C_{16}–C_{24}), and phytosphingosine and 2-hydroxy fatty acids (C_{22}–C_{24}), respectively. No branched paraffin chains were found in significant amounts. Sphingomyelins with trihydroxy long-chain bases and 2-hydroxy fatty acids found in jejunoileum were also detected in bovine kidney and have not been demonstrated before. These sphingomyelins from both kidney and jejunoileum showed a preferential combination of trihydroxy bases and fatty acids with very long chains (C_{22}–C_{24}).

Supplementary key words ceramides · thin-layer chromatography · gas–liquid chromatography · mass spectrometry

The small intestine from different mammalian species has been the object of several sphingolipid studies. Most of these investigations were concerned with the carbohydrate and fatty acid portions of glycosphingolipids (1, 2), but some work on the long-chain base composition of sphingolipids has also been done (3, 4).

The histological appearance of the mucosal layer from different parts of the digestive tract varies a great deal, and this undoubtedly reflects the great number of functions found in this organ (5). This was the reason for taking anatomically different parts of bovine digestive tract as the object for a correlative study of sphingolipids, and this paper is concerned with the molecular species of sphingomyelins.

Some years ago a novel molecular species of sphingomyelin containing sphingosine and 2-hydroxy fatty acids was found in bovine rennet stomach (6). Recently, another species was found in bovine kidney and jejunoileum mucosa,[1] having phytosphingosine in combination with 2-hydroxy fatty acids, a heretofore unknown molecular species of sphingomyelin.

The monoglycosylceramides, investigated previously (7), revealed several interesting differences between the regions with regard to both the carbohydrate and the ceramide moieties. One interesting finding was a molecular species with galactose, sphingosine, and 2-hydroxyhexadecanoic acid, which was present only in colon ascendens.

MATERIALS AND METHODS

Preparation of sphingomyelins

Sphingomyelins from the mucosal layer of bovine rennet stomach, duodenum, jejunoileum, and colon ascendens were prepared as described previously (6).

Enzymatic degradation of sphingomyelins

The phospholipid was degraded by phospholipase C treatment to yield ceramide and phosphorylcholine (8). From jejunoileum a larger quantity of sphingomyelins was degraded in order to isolate minor (less than 1%) ceramide fractions. The completeness of hydrolysis was checked by thin-layer chromatography.

Nomenclature: Sphingosine means 1,3-dihydroxy-2-aminooctadecene, and phytosphingosine means 1,3,4-trihydroxy-2-aminooctadecane. Dihydroxy base and trihydroxy base mean any base with two and three hydroxy groups, respectively. The shorthand designations used are explained in Table 1.

[1] Breimer, M. E., K.-A. Karlsson, and B. E. Samuelsson. Accepted for publication in *Lipids*.

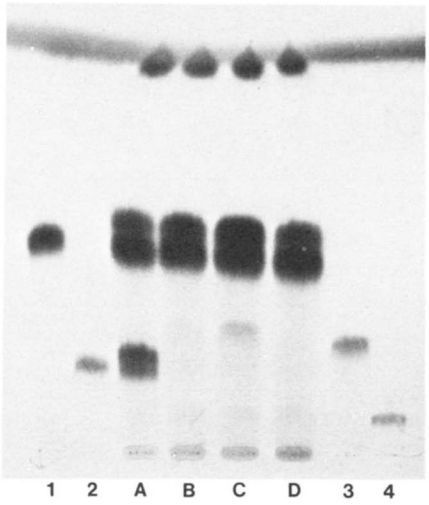

Fig. 1. Thin-layer chromatogram of ceramides derived from sphingomyelins of bovine rennet stomach (*A*), duodenum (*B*), jejunoileum (*C*), and colon ascendens (*D*). Reference compounds were synthetic ceramides of: sphingosine–octadecanoic acid (d18:1–18:0), lane 1; sphingosine–D-2-hydroxyoctadecanoic acid (d18:1–Dh18:0), lane 2; phytosphingosine–octadecanoic acid (t18:0–18:0), lane 3; and phytosphingosine–D-2-hydroxyoctadecanoic acid (t18:0–Dh18:0), lane 4. The thin-layer was silica gel G (Fluka), the solvent was chloroform–methanol 95:5 (v/v), and a copper acetate reagent (22) was used for detection.

Separation of total ceramides into subgroups

The different ceramide groups were separated using preparative thin-layer chromatography (8, 9) and column chromatography (6, 10) as described earlier.

Characterization of ceramides

The ceramides were characterized by thin-layer chromatography (9), using synthetic ceramides as references (a gift from I. Pascher of this department).

Direct inlet mass spectrometry of trimethylsilyl ethers of the intact ceramides was performed on an LKB 9000 or an MS 902 mass spectrometer as described earlier (11). The interpretation of the fragments was based on earlier published mass spectra of synthetic ceramides (12, 13).

The trimethylsilyl ethers of intact ceramides were analyzed by gas–liquid chromatography; the technical conditions are given in the legend to Fig. 3. The different peaks were identified by comparing the retention times with those of ceramides from bovine kidney that were identified by combined gas–liquid chromatography and mass spectrometry[2] in a manner similar to the procedures of Krivit and Hammarström (14) for ceramides of human platelets. The chromatograms were also compared with the mass spectra obtained by direct analysis of the ceramide fraction and with an earlier study of the long-chain base and fatty acid composition of sphingomyelins from rennet stomach (6).

[2] Breimer, M. E., K.-A. Karlsson, and B. E. Samuelsson. To be published.

RESULTS

The total amounts of sphingomyelins obtained from the mucosal layer of bovine rennet stomach, duodenum, jejunoileum, and colon ascendens were 9.1, 7.2, 6.9, and 8.8 mg/g dry tissue weight, respectively.

Thin-layer chromatography

The thin-layer chromatogram of the total ceramide fractions obtained by phospholipase hydrolysis of sphingomyelins is shown in **Fig. 1.** The enzyme preparation used in the present work has been shown not to liberate any free fatty acids or long-chain bases (8). The spots seen at the origin and at the solvent front in the figure are probably due to contaminating material of the phospholipase preparation. They were eliminated by preparative thin-layer chromatography and column chromatography. The major ceramide group in all fractions had the same mobility as ceramides consisting of dihydroxy long-chain bases in combination with normal fatty acids. This is shown by the large spots that have a mobility similar to synthetic ceramide with sphingosine and octadecanoic acid (Fig. 1). The double-band appearance of the spots is due to differences in fatty acid chain length, as ceramides having long (C_{16-18}) and very long (C_{20-24}) chain fatty acids are known to separate in the solvent system that was used (9).

In rennet stomach a slow-moving ceramide group was seen (Fig. 1, *A*); the lower band had an R_f value identical with that of a synthetic ceramide with sphingosine and D-2-hydroxyoctadecanoic acid (t18:0–Dh18:0) (Fig. 2, *E*), band that moved just ahead of the synthetic ceramide with phytosphingosine and octadecanoic acid (t18:0–18:0) is shown for jejunoileum (Fig. 1, *C*). In addition, there are some very faint bands in all of the ceramide fractions.

In order to identify minor ceramide components, a large batch of sphingomyelins from jejunoileum was degraded and the different ceramide groups were isolated using column chromatography and preparative thin-layer chromatography. The thin-layer chromatogram of the minor fractions is reproduced in **Fig. 2.** Of the three fractions obtained, one had a double band (Fig. 2, *C*) with the same mobility as the slow-moving ceramide group of rennet stomach (Fig. 2, *B*). The other two fractions had single bands, which migrated just in front of the reference species with phytosphingosine and octadecanoic acid (Fig. 2, *D*) and the reference species with phytosphingosine and D-2-hydroxyoctadecanoic acid (t18:0–Dh18:0) (Fig. 2, *E*), respectively.

Mass spectrometry

The different ceramide fractions obtained by preparative thin-layer and column chromatography were converted to trimethylsilyl ethers and subjected to direct-inlet mass spectrometry. The long-chain bases and fatty acids of

the sphingomyelins of rennet stomach have been characterized in detail before (6). Two groups of ceramides were found by thin-layer chromatography after phospholipase degradation (Fig. 1, *A*). The fast-moving group contained sphingosine as the predominant long-chain base. The C_{17} and C_{20} homologs of sphingosine were also present in small amounts. The predominant fatty acids were saturated normal C_{16}, C_{18}, C_{22}, and C_{24}. The slow-moving ceramide group also contained sphingosine as the major long-chain base and small amounts of the C_{17} homolog. The fatty acids were saturated 2-hydroxy C_{16}, C_{22}, C_{23}, and C_{24}.

The long-chain base of the total ceramide fraction from duodenum was exclusively sphingosine, as indicated by the mass spectrum. Only traces of the C_{17} homolog were present. No fragments corresponding to trihydroxy long-chain bases (phytosphingosine and related bases) were found. The predominant fatty acids were saturated normal C_{16}, C_{18}, C_{22}, and C_{24} and monounsaturated normal C_{24}. The absence of fragments corresponding to trihydroxy bases and hydroxy fatty acids in the spectrum of the total fraction was expected from the thin-layer plate (Fig. 1, *B*), where only two barely visible bands were seen behind the major spot.

From jejunoileum four different ceramide groups were obtained (Fig. 1, *C*, and Fig. 2, *C–E*). The major one contained sphingosine and saturated normal C_{16}, C_{18}, C_{22}, C_{23}, and C_{24} fatty acids. The ceramide group that on thin-layer chromatography migrated as a single band just in front of the synthetic ceramide with phytosphingosine and octadecanoic acid (see Fig. 1, *C*, and Fig. 2, *D*) was found to contain phytosphingosine as the main long-chain base together with small amounts of the C_{17} homolog. The major fatty acids were saturated normal C_{16}, C_{22}, C_{23}, and C_{24}. The third ceramide fraction had the same mobility as the slow-moving double band from rennet stomach (Fig. 2, *B* and *C*). It had a mass spectrum similar to that from rennet stomach, showing sphingosine and saturated 2-hydroxy C_{16}, C_{18}, C_{22}, and C_{24} fatty acids. The most polar ceramide group isolated from jejunoileum migrated as a single band just in front of the synthetic ceramide sample with phytosphingosine and D-2-hydroxyoctadecanoic acid (Fig. 2, *E*). The mass spectrum showed the presence of phytosphingosine together with 2-hydroxy C_{22}, C_{23}, and C_{24} fatty acids. This molecular species of ceramide derived from sphingomyelins was recently found in bovine kidney;[1] this is the first demonstration of trihydroxy long-chain bases combined with hydroxy fatty acids in sphingomyelins. Mass spectra and further details are presented elsewhere.[1]

Mass spectra of the total ceramide fraction from colon ascendens indicated sphingosine as the only long-chain base. There was no evidence for the existence of trihydroxy long-chain bases. The main fatty acids were saturated normal C_{16}, C_{18}, C_{22}, and C_{24}, together with a C_{24} monounsaturated acid.

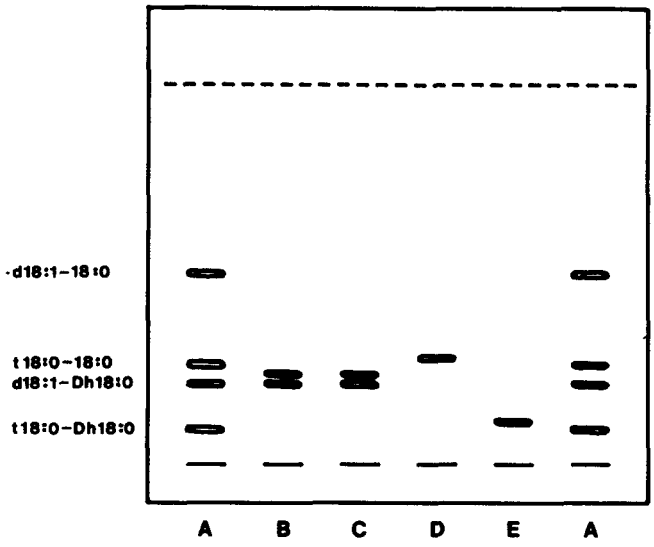

Fig. 2. Thin-layer chromatogram of minor ceramide groups isolated from sphingomyelins of bovine rennet stomach (*B*) and jejunoileum (*C, D,* and *E*). The shorthand designations (for explanation, see legend to Fig. 1) refer to a mixture of synthetic ceramides used as reference (*A*). The technical conditions were the same as indicated in Fig. 1.

Gas–liquid chromatography

The ceramide fractions discussed above were also analyzed as their trimethylsilyl derivatives by gas–liquid chromatography. The major molecular species are listed in **Table 1** and the chromatogram of the fraction from duodenum is shown in **Fig. 3.** The different peaks were identified by comparing the retention times with those of ceramides from bovine kidney sphingomyelins, which were analyzed by combined gas–liquid chromatography and mass spectrometry.[2] The identities of the peaks were further confirmed by the mass spectra of total ceramide fractions (direct-inlet analysis, see the results above).

There was a slight quantitative difference between the chromatographic peak distribution of ceramides of rennet stomach (Table 1) compared with the previously reported fatty acid composition (6). This is mainly due to the presence of small amounts of C_{17} and C_{20} homologs of sphingosine. The ceramides separate mainly according to the total number of carbon atoms in the molecules (see Fig. 3), and therefore two or more molecular species may be present in one peak. The use of mass spectrometric analysis of each ceramide peak may aid in the quantitation, but the excess bleeding from the column at the high temperature used makes it difficult to quantify small amounts of sample (intense background of the spectra). In all ceramide fractions except rennet stomach, the mass spectra of total ceramides showed only traces of sphingosine homologs; therefore, the values in Table 1 are fairly accurate. The presence of branched paraffin chains and the degree

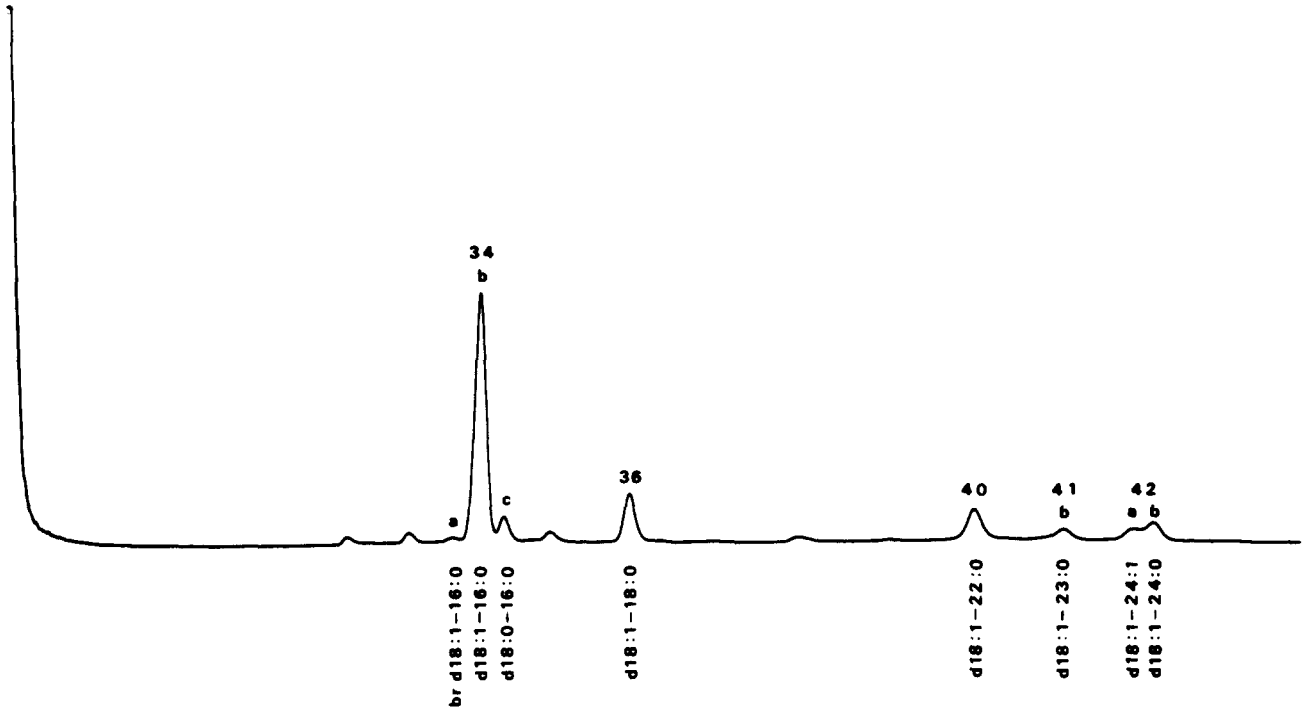

Fig. 3. Gas chromatogram of trimethylsilyl ethers of ceramides derived from sphingomyelins of mucosal layer of bovine duodenum. Peak numbers refer to Table 1. The stationary phase was 1% OV-1 (Applied Science Laboratories). The column temperature was kept isothermal at 260°C for 6 min and then raised to 310°C (0.5°C/min). The retention time for peak 34b was 40 min. The ceramides were separated according to total number of carbon atoms, degree of unsaturation, and branches, as indicated by shorthand designations below the peaks (see legend to Table 1).

of unsaturation are also indicated (see Fig. 3). In some fractions, dihydrosphingosine (d18:0) was found in small amounts, but only traces of branched-chain ceramides were present in all fractions.

DISCUSSION

The ceramides derived from sphingomyelins of different regions of bovine digestive tract showed a relatively simple

TABLE 1. Major ceramide species of sphingomyelins from different parts of bovine digestive tract

Peak No.[a] (Σ carbon atoms)	Major Ceramide Species					
	Rennet Stomach		Duodenum	Jejunoileum		Colon Ascendens
	d–n (75)[b]	d–h (25)	d–n	d–n (98)	t–n (2)	d–n
C33	d17:1–16:0 (4)	d17:1–h16:0 (4)				
C34 b	d18:1–16:0 (49)	d18:1–h16:0 (38)	d18:1–16:0 (49)	d18:1–16:0 (40)		d18:1–16:0 (50)
c	d18:0–16:0 (3)		d18:0–16:0 (6)	d18:0–16:0 (7)	t18:0–16:0 (7)	
C35	d18:1–17:0 (3)[c]		d18:1–17:0 (4)[c]	d18:1–17:0 (3)[c]		
C36	d18:1–18:0 (9)[d]		d18:1–18:0 (11)	d18:1–18:0 (13)		d18:1–18:0 (10)
C40	d18:1–22:0 (7)	d18:1–h22:0 (14)	d18:1–22:0 (12)	d18:1–22:0 (11)	t18:0–22:0 (18)	d18:1–22:0 (10)
C41 a					t18:0–23:1 (4)	
b	d18:1–23:0 (4)[e]	d18:1–h23:0 (16)	d18:1–23:0 (4)	d18:1–23:0 (7)	t18:0–23:0 (14)	d18:1–23:0 (4)
C42 a		d18:1–h24:1 (3)	d18:1–24:1 (5)		t18:0–24:1 (9)	d18:1–24:1 (5)
b	d18:1–24:0 (8)	d18:1–h24:0 (12)	d18:1–24:0 (6)	d18:1–24:0 (7)	t18:0–24:0 (34)	d18:1–24:0 (6)
C43 a					t18:0–25:1 (5)	
b					t18:0–25:0 (3)	

[a] Peak numbers refer to the gas–liquid chromatographic analysis (Fig. 3).
[b] Numbers in parentheses represent percentage composition. Ceramide species less than 3% are not included. d, dihydroxy long-chain bases; t, trihydroxy long-chain bases; n, normal fatty acid; h, hydroxy fatty acid.
[c] And/or d17:1–18:0 (number of carbon atoms:number of double bonds).
[d] And d20:1–16:0.
[e] And/or d17:1–24:0.

pattern (Fig. 1). The major ceramide group in all fractions was composed of dihydroxy long-chain bases and normal fatty acids having both long and very long chains (Table 1). The major molecular species was sphingosine combined with hexadecanoic acid; this gave rise to the lower part of the major double band seen in Fig. 1. The upper band probably contained fatty acids with longer chains (8, 9). Only two additional ceramide groups were clearly seen on the plates. In rennet stomach (Fig. 1, *A*) a ceramide with dihydroxy long-chain bases and an equal distribution of long and very long chain 2-hydroxy fatty acids was relatively abundant. In jejunoileum a single band was found that was due to trihydroxy long-chain bases and normal fatty acids with longer chains (Table 1).

This relatively simple pattern should be compared with the complexity of monoglycosylceramides of the same organ (7). The amount of glucosylceramides was about five times higher in rennet stomach than in colon ascendens, but the amounts of galactosylceramides were fairly constant in all regions. In rennet stomach and colon ascendens, sphingosine was the predominant long-chain base, but in small intestine, sphingosine and phytosphingosine were present in approximately equal amounts. The fatty acids present in all regions were apparently only 2-hydroxy fatty acids with longer chains except in colon ascendens, where, in addition, a specific molecular species consisting of galactose, sphingosine, and 2-hydroxyhexadecanoic acid was found in large amounts.

Sphingomyelins from rat intestine have been shown to contain both dihydroxy and trihydroxy long-chain bases (3). Trihydroxy bases have been detected in mucosal sphingolipids of rat, rabbit, and dog intestine (4). Branched paraffin chains have been found in both dihydroxy and trihydroxy long-chain bases of sphingomyelins from bovine kidney (10, 15). Sphingomyelins isolated from bovine milk (16) show a complex long-chain base pattern, having saturated and unsaturated dihydroxy bases with both straight and branched paraffin chains (for a review on long-chain bases, see Ref. 17). In bovine digestive tract there were only traces of branched chains, as indicated by the gas chromatogram.

A preferential combination of trihydroxy bases and fatty acids with very long chains has been found in bovine kidney sphingomyelins (15).[2] The digestive tract is similar in this respect, with dihydroxy bases combined with both long and very long chains, but trihydroxy bases are found mainly in combination with very long chains.

The existence of phytosphingosine in mammalian sphingolipids has been known for a long time (17, 18). The presence of sphingomyelins with trihydroxy long-chain bases and 2-hydroxy fatty acids in small amounts in jejunoileum and also in bovine kidney is notable. This means that the complexity of the lipophilic part of mammalian phosphosphingolipid is as great as for glycosphingolipid, where all combinations of trihydroxy and dihydroxy bases with normal and 2-hydroxy fatty acids have been known for some time (17, 19). Whether this relatively polar molecular species is part of a separate membrane unit can at present be only the subject of speculation. It may be noted that a unique combination of trihydroxy base and 2-hydroxy fatty acid has been found in phosphosphingolipid (ceramide 2-*N*-methylaminoethylphosphonate) of an invertebrate (20).

Too little is known about the membrane lipid composition of different parts of the digestive tract or about physicochemical properties of sphingolipids (21) to allow conclusions about the present findings. However, the distinct regional differences found for both the monoglycosylceramides (7) and the sphingomyelins should be related to functional membrane characteristics, such as permeability and transport properties.

The author is indebted to K.-A. Karlsson for advice and for the samples of sphingomyelins. The work was supported by grants from the Swedish Medical Research Council (project 03X-3967) to K.-A. Karlsson and from the Medical Faculty, University of Göteborg.

Manuscript received 29 August 1974; accepted 14 January 1975.

REFERENCES

1. Forstner, G. G., and J. R. Wherrett. 1973. Plasma membrane and mucosal glycosphingolipids in the rat intestine. *Biochim. Biophys. Acta.* **306:** 446–459.
2. McKibbin, J. M. 1969. The composition of the glycolipids in dog intestine. *Biochemistry.* **8:** 679–685.
3. Yurkowski, M., and B. L. Walker. 1970. The long chain bases in rat mucosal sphingolipids. *Biochim. Biophys. Acta.* **218:** 378–380.
4. Okabe, K., R. W. Keenan, and G. Schmidt. 1968. Phytosphingosine groups as quantitatively significant components of the sphingolipids of the mucosa of the small intestines of some mammalian species. *Biochem. Biophys. Res. Commun.* **31:** 137–143.
5. Ganong, W. F. 1971. Gastrointestinal function. *In* Medical Physiology. Lange Medical Publications, Los Altos, Calif. 346–374.
6. Karlsson, K.-A., K. Nilsson, B. E. Samuelsson, and G. O. Steen. 1969. The presence of hydroxy fatty acids in sphingomyelins of bovine rennet stomach. *Biochim. Biophys. Acta.* **176:** 660–663.
7. Breimer, M. E., K.-A, Karlsson, and B. E. Samuelsson. 1974. The distribution of molecular species of monoglycosylceramides (cerebrosides) in different parts of bovine digestive tract. *Biochim. Biophys. Acta.* **348:** 232–240.
8. Karlsson, K.-A. 1968. Enzymatic hydrolysis of sphingomyelins: use in structure analysis. *Acta Chem. Scand.* **22:** 3050–3052.
9. Karlsson, K.-A., and I. Pascher. 1971. Thin-layer chromatography of ceramides. *J. Lipid Res.* **12:** 466–472.
10. Karlsson, K.-A., B. E. Samuelsson, and G. O. Steen. 1973. Detailed structure of sphingomyelins and ceramides from

different regions of bovine kidney with special reference to long-chain bases. *Biochim. Biophys. Acta.* **316:** 336–362.
11. Karlsson, K.-A., B. E. Samuelsson, and G. O. Steen. 1973. Separation of monoglycosylceramides (cerebrosides) of bovine kidney into subgroups and characterization by mass spectrometry. *Biochim. Biophys. Acta.* **306:** 317–328.
12. Samuelsson, K., and B. Samuelsson. 1970. Gas chromatographic and mass spectrometric studies of synthetic and naturally occurring ceramides. *Chem. Phys. Lipids.* **5:** 44–79.
13. Hammarström, S. 1970. Gas–liquid chromatography–mass spectrometry of synthetic ceramides containing phytosphingosine. *J. Lipid Res.* **11:** 175–182.
14. Krivit, W., and S. Hammarström. 1972. Identification and quantitation of free ceramides in human platelets. *J. Lipid Res.* **13:** 525–530.
15. Karlsson, K.-A., and G. O. Steen. 1968. Studies on sphingosines. XIII. The existence of phytosphingosine in bovine kidney sphingomyelins. *Biochim. Biophys. Acta.* **152:** 798–800.
16. Morrison, W. R. 1969. Polar lipids in bovine milk. 1. Long-chain bases in sphingomyelin. *Biochim. Biophys. Acta.* **176:** 537–546.
17. Karlsson, K.-A. 1970. Sphingolipid long-chain bases. *Lipids.* **5:** 878–891.
18. Karlsson, K.-A. 1964. Studies on sphingosines. 7. The existence of C_{18}- and C_{20}-phytosphingosine in animal tissues. *Acta Chem. Scand.* **18:** 2397–2398.
19. Karlsson, K.-A. 1970. On the chemistry and occurrence of sphingolipid long-chain bases. *Chem. Phys. Lipids.* **5:** 6–43.
20. Hayashi, A., and F. Matsuura. 1973. 2-Hydroxy fatty acid- and phytosphingosine-containing ceramide 2-*N*-methylaminoethylphosphonate from *Turbo cornutus*. *Chem. Phys. Lipids.* **10:** 51–65.
21. Abrahamsson, S., I. Pascher, K. Larsson, and K.-A. Karlsson. 1972. Molecular arrangements in glycosphingolipids. *Chem. Phys. Lipids.* **8:** 152–179.
22. Fewster, M. E., B. J. Burns, and J. F. Mead. 1969. Quantitative densitometric thin-layer chromatography of lipids using copper acetate reagent. *J. Chromatogr.* **43:** 120–126.