PHYTOSPHINGOSINE GROUPS AS QUANTITATIVELY SIGNIFICANT COMPONENTS OF THE SPHINGOLIPIDS OF THE MUCOSA OF THE SMALL INTESTINES OF SOME MAMMALIAN SPECIES.[1]

Kazuhiko Okabe, Roy W. Keenan and Gerhard Schmidt
Department of Biochemistry and New England Medical Center Hospitals, Tufts University School of Medicine, Boston, Massachusetts.

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We found that the lipid bases of the small intestines of fasted rats differed from those of other tissues by the presence of a fraction that moved more slowly than C_{18}-dihydrosphingosine on thin-layer (TL) chromatograms in the solvent mixture of Sambasivarao and McCluer (1963). Observations which will be described in this report leave little doubt that this fraction consists, at least predominantly, of C_{18}-phytosphingosine.

The occurrence of phytosphingosine in sphingolipids of some human tissues, e. g. of hair, and in the ceramide hexosides of kidney has been observed (Karlsson 1964, Michalec and Kolman 1966, Karlsson and Mårtensson 1968). Phytosphingosine was not found in the sphingomyelin fraction of human kidney.

Materials and Methods. Adult rats (250 g) were fasted for 72 hours, dogs and rabbits for 24 hours before they were sacrificed.

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The mucosa of the small intestines which had been rinsed with Ringer's solution was prepared by gentle scraping of the loops, and was homogenized immediately in 20 volumes of chloroform-methanol mixture 2:1 (v/v) for the extraction of the lipids according to Folch et al. (1957).

The fractionation of the lipids by TL-chromatography was carried out according to Wüldner et al. (1962). Owing to the large distances between the spots of sphingomyelin, cerebrosides and ceramides, this technique facilitated the subsequent elution of the individual sphingolipid fractions.

The alkali-stable lipid fraction which includes all aphingolipids present in the extract was prepared according to Dawson et al (1962).

The methanolysis of the total lipids or of individual sphingolipid fractions eluted from the TL-chromatograms and the subsequent separation of the lipid bases by TL-chromatography was carried out as described in an earlier communication (Schmidt et al., 1966). Under these conditions, sphingosine is practically completely converted to O-methyl derivatives, but neither dihydrosphingosine nor phytosphingosine are methylated to more than negligible extents. This was demonstrated by TL-chromatography of pure samples of the latter two bases as well as by gas-liquid chromatography of the fatty aldehydes formed from the bases by periodate oxidation before and after their treatment under the condition s of methanolysis. (Sweeley and Moscatelli 1959). A generous quantity of pure phytosphingosine was kindly given to us by Dr. H. E. Carter and Dr. A. Kisic, University of Illinois, Urbana, Illinois.

The lipid bases were quantitatively determined according to Schmidt et al. (1966) on the basis of the ninhydrin reaction. The absorbance of the ninhydrin color obtained with 0.1 mg phytosphingosine and

measured in 25 ml was found $A_{1\ cm}^{570\ m\mu}$=0.154. (The corresponding absorbancies with 0.1 mg dihydrosphingosine and with 0.1 mg sphingosine were respectively 0.135 and 0.245).

The trimethylsilylation of the lipid bases and the subsequent separation and identification of the derivatives by gas-liquid chromatography were carried out according to Carter and Gaver (1967).

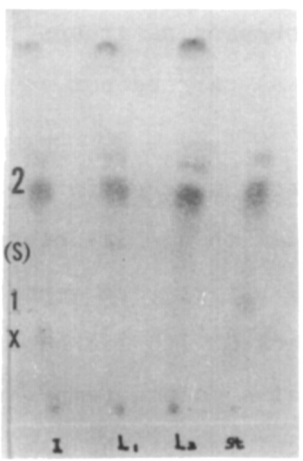
The periodate oxidation of the lipid bases and the identification of the lipid aldehydes by gas-liquid chromatography were performed according to Sweeley and Moscatelli (1959), the simultaneous oxidation of the lipid bases by periodate and permanganate to fatty acids and the identification of the latter according to Greene, Kaneshiro and Law (1965).

The periodate oxidation of the phytosphingosine groups in sphingolipids without preceding methanolysis was carried out as described by Carter, Gaver and Yu (1966).

The analyses by gas-liquid chromatography were performed with a Barber-Colman Model 10 apparatus equipped with a ^{90}Sr-detector. The fatty aldehydes and the fatty acid methyl esters were separated on 6 ft ×4 mm I.D. U-shaped glass columns packed with 15% butanediol succinate on Chromosorb W (80-100 mesh) at a column temperature of 175°, the trimethylsilyl derivatives on columns of the same dimensions which were packed with 3.8% Silicone rubber (UC W98) on Diatoport S (Hewlett-Packard Co., Avondale, Pennsylvania) at a column temperature of 192°.

Experimental. Figure 1 shows a representative TL-chromatogram of the lipid bases of the sphingolipids of the small intestines (I) and the liver (L_1 and L_2) of an adult rat. For a reference chromatogram, a solution of the mixture of lipid bases obtained by methanolysis of

the sphingolipids of monkey spinal cord was applied (St). The intense clusters of the O-methyl sphingosine spots (2) are present on all chromatograms of Figure 1 whereas spots corresponding to dihydrosphingosine (1) are barely discernible on the chromatograms of the lipid bases of the two rat tissues. On the other hand, the chromatogram of the intestinal lipid bases (I) contains a slow-moving spot (X) which is absent from the reference chromatogram (St) and from the chromatogram L_1 of the lipid bases of liver, and which is only very faint on the chromatogram L_2 in which twice the amount of lipid bases used for L_1 was applied. (The three spots at the solvent front of I, L_1 and L_2 are not ninhydrin-positive.)



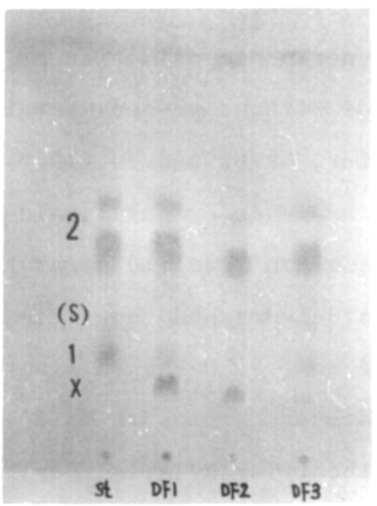


Figure 1. TL-chromatogram of the lipid bases of the sphingolipids of the small intestines (I) and the liver (L_1, L_2) of an adult rat. Reference chromatogram (St) of total lipid bases of monkey spinal cord. The amounts spotted correspond to 165 µg lipid-P in I, 90 µg lipid-P in L_1 and 180 µg lipid-P in L_2.

Figure 2. TL-chromatogram of the lipid bases obtained respectively from sphingomyelin (DF_1), cerebrosides (DF_2) and ceramides (DF_3) of dog jejunal mucosa. (0.2 g wet weight, 0.018 g dry weight) Reference chromatogram (St) as in Figure 1. The spots marked (S) migrated at the position characteristic for nonmethylated sphingosine.

Observations demonstrating C_{18}-phytosphingosine as the principal constituent of fraction X of the lipid bases of dog small intestines. The lipid base obtained by elution of spot X from the TL-chromatograms moved as a single spot at the same rate as C_{18}-phytosphingosine during rechromatography on thin-layer plates. This result was obtained with fraction X of intestinal mucosa of all species analyzed.

Gas-liquid chromatography of the trimethylsilyl derivatives of fraction X from dog small intestines yielded one symmetrical peak the retention time of which was identical with that of trimethylsilyl phytosphingosine. Mixtures of the trimethylsilyl derivative of fraction X and of trimethylsilyl phytosphingosine yielded a single peak on gas-liquid chromatograms.

Gas-liquid chromatograms of the aldehydes formed during the periodate oxidation of fraction X from dog intestinal mucosa showed one major peak (85%) of pentadecanal and two small peaks of hexa- and heptadecanal. When the periodate reaction was carried out in presence of alkaline permanganate, the corresponding fatty acids were obtained in the expected quantities.

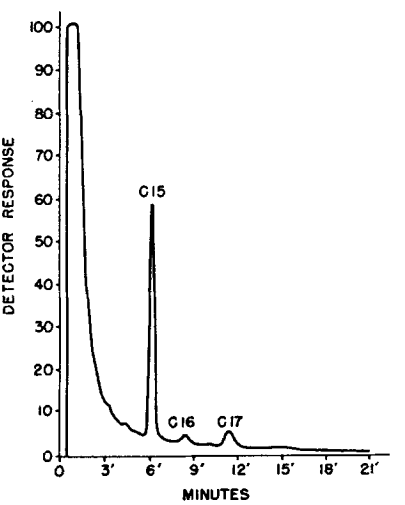


Figure 3. Gas-liquid chromatogram of the aldehydes formed from fraction X (see Fig. 1) of the lipid bases of dog intestinal mucosa. (15% butane diol succinate polyester on Chromosorb W , (80-100 mesh), 175°, 20 p s. i. of argon.

Periodate oxidation of the non-methanolysed, alkali-stable lipid fraction of dog intestinal mucosayielded a major peak of pentadecanal and small peaks of hexadecanal and heptadecanal.

Representative values of the concentrations of the lipid bases obtained by methanolysis of the lipids of the mucosa of the small intestines of rat, rabbit and dog are given in Table I.

Table I
Concentrations of the Lipid Base Groups of the Sphingolipids of the Mucosa of the Small Intestines of Some Mammalian Species

Species	Total Lipid Bases	Dihydro-sphingosine	Phyto-sphingosine	Lipid-P
	micromoles per gram of moist tissue			
Rat	1.60 (0.16)*	0.05	0.325	10.3
Rabbit	2.12 (0.13)*	0.14	0.568	17.4
Dog	1.85 (0.13)* (0.15)**	0.12	0.341	14.7

*The figures in brackets are the molar ratios **total** lipid bases:lipid-P
** The figure is the molar ratio of total lipid-bases:lipid-P in dog intestinal mucosa as calculated from data reported by McKibbin, Meltzer and Spiro (1961).

In the mucosae of the stomach and of the colon of dogs, phytosphingosine accounted for much smaller percentages of the total lipid bases than in that of the small intestines.

Discussion. Vance, Shook and McKibbin (1966) described the isolation from dog intestinal mucosa of glycosphingolipid compounds which differed from the corresponding sphingolipid constituents of other tissues with regard to certain characteristic structural features such as the chain lengths of the fatty acid groups and-in the case of the major ganglioside constituent-the amount of sialyl group per molecule.

Possibly, the unusually high ratio phytosphingosine:total lipid bases **might** represent likewise a tissue-specific feature of the sphingolipids of the small intestines.

On the other hand, a direct exogenous origin of intestinal phytosphingosine groups by absorption of plant sphingolipids cannot be excluded at present although the animals studied in this investigation had been fasted before they were sacrificed. Owing to the slow metabolic turnover of many complex sphingolipids, the daily absorption of small quantities of such compounds might result eventually in appreciable accumulations of phytosphingosine-containing sphingolipids in the intestinal mucosa.

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