Human Plasma Sphingomyelins.* (29097)

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The excellence of thin-layer chromatography as a method for the small-scale separation of sphingomyelin free from contamination with phosphatidyl choline or lysophosphatidyl choline has been noted(1,2). We have noticed that on silica gel plates, with a suitable solvent, the sphingomyelin region of fresh lipids from various animal sources very often appears as a double spot. Habermann *et al*(3) have reported this finding for human plasma phospholipids, and we have noted it also for sphingomyelin from human chylomicrons(2), sheep, hog and steer sera, and egg yolk(4). We wish to report some preliminary investigations into the nature of these 2 separating components.

Methods and results. Properties of sphingomyelin components. Total lipids of normal human plasma were extracted and washed by the procedure of Folch *et al*(5). The lipids, dissolved in chloroform, were applied as single spots to plates coated with Silica Gel G (E. Merck A. G., Darmstadt) 250 μ thick, and developed in a mixture of chloroform-methanol-water 80/35/5 (v/v). After visualization of lipids by spraying with 50% (v/v) sulfuric acid and charring at 250°, two characteristically-shaped spots were seen, close together, in the sphingomyelin region, between the large phosphatidyl choline spot and the lysophosphatidyl choline region (Fig. 1, position 1). It was noted that the 2 spots tend to coalesce on heavy loading, and that separation is not obtained in solvent systems that are appreciably less polar than the one described. By the use of spray reagents it was shown for both components that phosphorus was present but free amino groups absent. Habermann *et al* showed also that plasmalogens and ester structures were absent from both spots.

Treatment of plasma lipids with methanolic sodium methoxide in the cold resulted in rapid hydrolysis of the phosphatidyl ethanolamine, phosphatidyl choline and lysophosphatidyl choline, but both components of the sphingomyelin region were resistant to alkaline hydrolysis (Fig. 1) as would be expected for compounds containing amide-linked fatty acids(6). When plasma lipids were placed on a column of diethylaminoethyl cellulose acetate and eluted with 7/1 (v/v) chloroform-methanol, the only phospholipid retained (as judged by thin-layer chromatography of the eluate) was phosphatidyl ethanolamine. The elution of both of the components of the sphingomyelin region under these conditions suggested that inositol phospholipids may be excluded(7).

Preparation of sphingomyelin components. Samples of the 2 components were prepared by thin-layer chromatography of plasma total phospholipids (4 mg per plate, applied as a streak) on long plates (26 × 20 cm) coated with a 400 μ layer of Silica Gel G, and using chloroform-methanol-water 80/35/5 (v/v) as solvent (Fig. 2). Visualization was by iodine vapor, using a masking technique(2). The 2 silicic acid regions, containing respectively the more-polar and the less-polar sphingomyelin components, were scraped off separately and the lipids eluted in small columns with methanol. Thin-layer chromatography of the eluates indicated that very little cross-contamination was present, since each showed a single spot only, on visualization. Lecithin was entirely absent from both fractions.

Rather more phosphorus was present in the upper, less-polar region than in the lower, the ratio being 1:0.87. In the work of Habermann *et al*(3), this ratio was found to be 1:0.86.

Amide-bound fatty acids. Methyl esters of fatty acids present in each component were prepared by transmethylation in a closed vessel using 5% (v/v) sulfuric acid in methanol and heating at 70° for 6 hours. These

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conditions had earlier been found to give complete methylation of sphingomyelin acids, without loss of unsaturated acids. Gas-liquid chromatography showed a marked difference in distribution of the major fatty acids in the two components (Table I).

Long-chain bases. The long-chain bases present in our 2 separated components were isolated and examined by thin-layer chromatography using the procedures of Sambasivarao and McCluer(8). Three ninhydrin-positive spots were obtained for both components and the chromatogram suggested that there was a similar distribution of long-chain bases within the 2 sphingomyelin regions.

Discussion. It seems clear that in existing estimates of the phospholipid composition of human plasma, based on column chromatography, for instance that of Nelson(9), the 2 components we have studied have been included together as sphingomyelin. Our observations suggest that both of the materials separating during thin-layer chromatography have properties appropriate for sphingomyelins, but markedly different fatty acid compositions. It is clear that separation of the two regions is not due to differences in degree of unsaturation of the constituent acids.

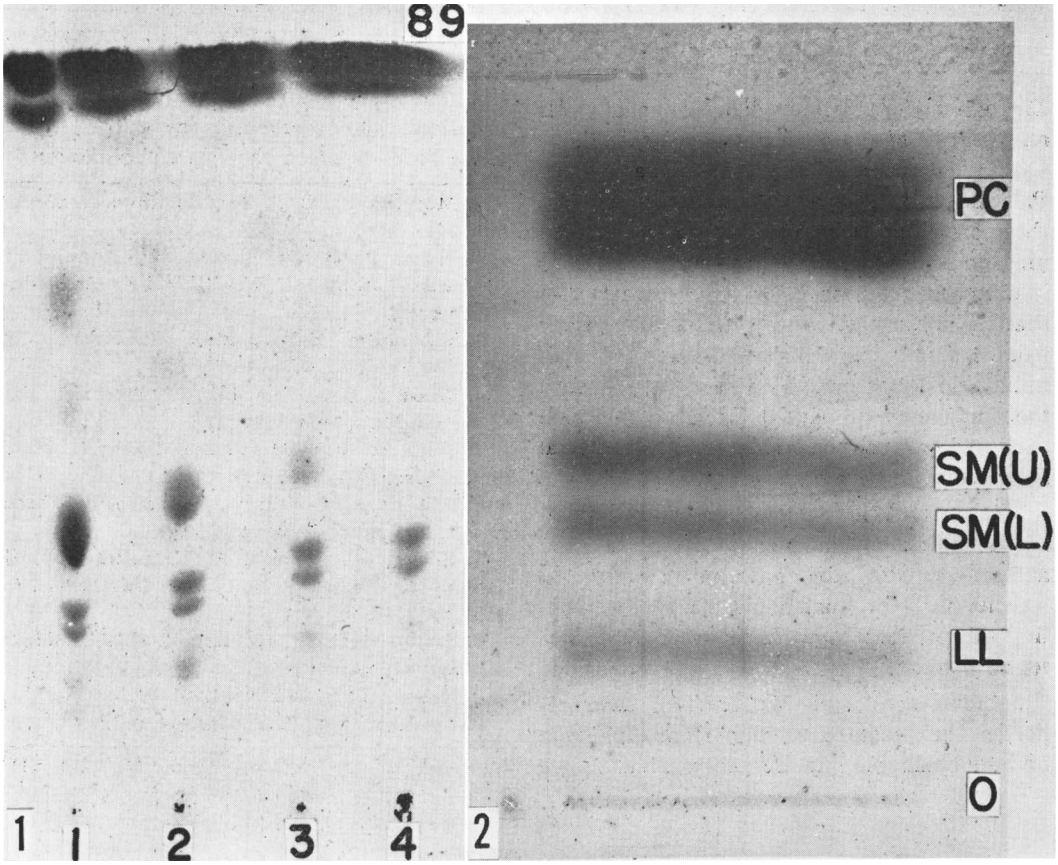

FIG. 1. Thin-layer chromatogram showing resistance of the 2 sphingomyelin components of human plasma lipids to alkaline hydrolysis with methanolic sodium methoxide. 1, phospholipids of plasma before treatment; 2, two min after treatment with methoxide; 3, five min after treatment; 4, fifteen min after treatment: the 2 sphingomyelin components remain intact, although hydrolysis of the other plasma phospholipids is almost complete.

FIG. 2. Separation obtained of 2 components of the sphingomyelin region of human plasma phospholipids, by thin-layer chromatography. Visualization was by spraying with 50% (v/v) sulfuric acid and heating at 250°. *PC*, phosphatidyl choline; *SM(U)*, upper, less-polar component of sphingomyelin region; *SM(L)*, lower, more-polar component of sphingomyelin region; *LL*, lysophosphatidyl choline region; *O*, origin.

TABLE I. Major Fatty Acids Present in the Two Components of Sphingomyelin from Human Plasma.

Fatty acid*	Mass % of total methyl esters: Upper, less-polar component	Lower, more-polar component
16:0	13.6	65.5
18:0	6.5	11.6
18:1	8.6	5.1
18:2	3.1	1.7
20:0	3.5	2.2
22:0	14.8	1.8
23:0	7.2	.8
24:0	15.1	2.2
24:1	20.8	.0

* The number preceding the colon denotes the chain length, and the number following it, degree of unsaturation of the fatty acid. Analyses were made by gas-liquid chromatography on 25% diethylene glycol succinate polyester at 180° with a Barber-Colman model 10 instrument(2).

There is a more striking distribution of acids on the basis of chain length, since acids above C_{20} are concentrated in the less-polar region. Palmitic acid, however, while concentrated in the more-polar component, is present in significant amount in the other component. This is in agreement with Nelson's observation(9) that the sphingomyelin of plasma eluted early from a silicic acid column contains less palmitic acid but more C_{23} and C_{24} acids than the sphingomyelin eluted later. His elution curve showed no fractionation of the total sphingomyelin region, however.

The fatty acid composition of our combined sphingomyelin components is in good agreement with the pattern reported by Sweeley(10) for human plasma sphingomyelin, except that very little oleic acid was found by him.

Sambasivarao and McCluer(8) have reported the presence of more than one type of long-chain base in human plasma. Since the distribution of long-chain bases in each component appeared to be similar, it may be that fatty acid content alone accounts for the separation of plasma sphingomyelin into 2 fractions; but the possibility remains that the long-chain bases are different in the intact phospholipids, but reach the same equilibrium composition during their isolation by hydrolysis(8).

Summary. The sphingomyelin region of human plasma lipids, entirely free from lecithin, has been fractionated into two components by thin-layer chromatography. Both components had properties characteristic of sphingomyelins, but markedly different fatty acid patterns, acids above C_{20} being concentrated in the less-polar material. On hydrolysis each component appeared to yield several different long-chain bases, but no striking difference was observed in the distribution of these bases between the two components.

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