[14] J. J. FOX, D. V. PRAAG, I. WEMPEN, I. L. DOERR, L. CHEONG, J. E. KNOLL, M. L. EIDINOFF, A. BENDICH AND G. B. BROWN, *J. Am. Chem. Soc.*, 81 (1959) 178.
[15] R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 52 (1952) 552.
[16] R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 45 (1949) 294.
[17] L. I. HECHT, P. C. ZAMECNIK, M. L. STEPHENSON AND J. F. SCOTT, *J. Biol. Chem.*, 233 (1958) 954.

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## Isolation and identification of lysolecithin from lipid extracts of normal human serum

The major phospholipids of human plasma eluted from silicic acid columns were recently reported by HIRSCH AND AHRENS[1] to be cephalins, lecithin, and sphingomyelin in that order. PHILLIPS[2,3] has reported the isolation of lysolecithin from human serum using the silicic acid chromatography technique of LEA, RHODES AND STOLL[4].

Silicic acid columns were prepared according to the specifications of HIRSCH AND AHRENS[1] and maintained at 25° for all runs. Serum was extracted with 20 vol. 2:1 (v/v) chloroform–methanol, washed with distilled water, evaporated to dryness under nitrogen, and applied to columns in petroleum ether. Omission of the water washing, storage of serum or the lipid extracts up to one week at 4°, or the use of siliconized glassware did not affect the results. The recovery of lipid phosphorus from the extracts was 88.2–97.2 %. Glycerides and free and esterified cholesterol were eluted with 150 ml diethyl ether.

Fig. 1 shows the three peaks obtained by elution of the phospholipids with 500 ml 1:4 (v/v) chloroform–methanol when 360-drop fractions were collected using an automatic fraction collector. The recovery of total lipid phosphorus was from 90–101%.

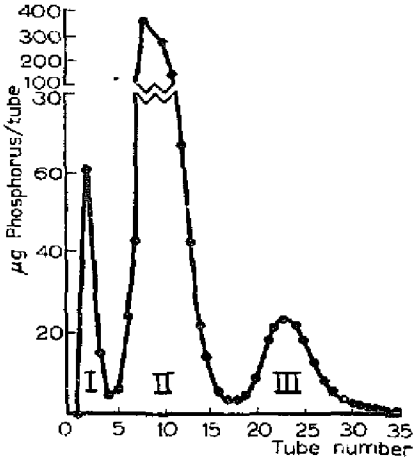


Fig. 1. Silicic acid chromatography of phospholipid from 15 ml normal human serum. Chloroform-methanol (1:4, v/v) as eluant with 360-drop fractions. Peak I, phosphatidylethanolamine + phosphatidylserine; Peak II, lecithin + sphingomyelin; Peak III, lysolecithin.

Peak I showed the presence of glycerol[5], a ninhydrin-positive reaction, and the absence of choline[6]. The carboxylic acid ester[7]/phosphorus[8] ratio was 2.01. Paper chromatography in three solvent systems[9–11] revealed the presence of phosphatidylethanolamine and phosphatidylserine. These could be separated by elution of the column with 200 ml 7:1 (v/v) chloroform–methanol followed by 200 ml 4:1 (v/v) chloroform-methanol. The Peak I material represented 6.4 % of the total lipid phosphorus and was present to the extent of 0.63 mg P/100 ml serum.

Peak II contained glycerol, but was negative to ninhydrin. The carboxylic acid ester/phosphorus ratio was 1.57 and the choline/phosphorus ratio was 0.73. Infrared spectra showed bands (1710, 1625, 1535, 1460 and 1170 $cm^{-1}$) which were compatible with lecithin (1710, 1460, 1170 $cm^{-1}$) and sphingomyelin (1625, 1535, 1460 $cm^{-1}$). Both

sphingomyelin and lecithin were present in paper chromatograms of Peak II material. Since sphingomyelin did not give a carboxylic ester reaction by the method of RAPPORT AND ALONZO[7], the lecithin content was estimated to be 78.5 % of the peak II phosphorus and sphingomyelin to represent 21.5 %. On this basis, lecithin had a carboxylic ester/phosphorus ratio of 2.00 and a choline/phosphorus ratio of 0.93. Lecithin thus represented 66.4 % of the total phosphorus or 6.54 mg P/100 ml serum, and sphingomyelin constituted 18.4 % of the total phosphorus or 1.79 mg P/100 ml serum.

The Peak III material contained glycerol but was ninhydrin-negative. The carboxylic ester/phosphorus ratio was 1.11 and the choline/phosphorus ratio was 1.17. The material was hemolytic to 2 ml of a 1 % suspension of washed human-red-blood cells in isotonic saline in an amount of 0.010 $\mu$mole. Paper chromatography revealed a spot which had the same mobility as the material produced by the action of pancreatic phospholipase A[12] on synthetic lecithin or on the peak II material. Peak III was resistant to the action of phospholipase A. The infrared spectrum was similar to a lecithin standard with the exception of a marked elevation of the 1355 m$\mu$ peak. This is suggestive of a free hydroxyl group. The Peak III material was identified as lysolecithin and represented 8.9 % of the total lipid phosphorus or 0.88 mg P/100 ml serum.

By gas-liquid partition chromatography the fatty acid compositions of the lecithin and lysolecithin were determined. The fatty acids of lecithin were 53.4 % saturated and 46.6 % unsaturated while the fatty acids of lysolecithin were 76.5 % saturated and 23.5 % unsaturated.

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[1] J. HIRSCH AND E. H. AHRENS, JR., *J. Biol. Chem.*, 233 (1958) 311.
[2] G. B. PHILLIPS, *Proc. Natl. Acad. Sci. U.S.*, 43 (1957) 566.
[3] G. B. PHILLIPS, *Biochim. Biophys. Acta*, 29 (1958) 594.
[4] C. H. LEA, D. N. RHODES AND R. D. STOLL, *Biochem. J.*, 60 (1955) 353.
[5] E. VAN HANDEL AND D. B. ZILVERSMIT, *J. Lab. Clin. Med.*, 50 (1957) 152.
[6] D. GLICK, *J. Biol. Chem.*, 156 (1944) 643.
[7] M. M. RAPPORT AND N. ALONZO, *J. Biol. Chem.*, 217 (1955) 193.
[8] E. J. KING, *Biochem. J.*, 26 (1932) 292.
[9] J. W. DIECKERT, W. B. CARNEY, R. L. ORY AND N. J. MORRIS, *Anal. Chem.*, 30 (1958) 1442.
[10] R. F. WITTER, G. V. MARINETTI, A. MORRISON AND L. HEICKLIN, *Arch. Biochem. Biophys.*, 68 (1957) 15.
[11] G. V. MARINETTI AND E. STOTZ, *Biochim. Biophys. Acta*, 21 (1956) 168.
[12] V. GRONCHI, *Sperimentale*, 90 (1936) 223.

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