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An improved method for separating the products of lecithin and lysolecithin catabolism

Enzymes that catalyze the deacylation of lecithin (3-*sn*-phosphatidylcholine) and lysolecithin (1- or 2-acyl-*sn*-glycero-3-phosphorylcholine) have been demonstrated in a number of mammalian tissues[1–6]. Further catabolism of glycero-3-phosphorylcholine (GPC) to glycero-3-phosphate and choline[7–9], together with the oxidation or phosphorylation of the newly formed choline[10], can also occur.

Previous methods for separating the hydrolysis products of lecithin or lysolecithin by ion-exchange chromatography[4,11] have produced three fractions corresponding to GPC, phosphorylcholine (PC), and choline plus betaine. The present report describes a method for chromatographically separating GPC, PC, betaine, and choline into distinct fractions. The results of studies which used this method to investigate the metabolism of lysolecithin *in vivo* will be reported separately.

Materials and methods

Dowex 50W (H^+ form, 100–200 mesh), glycerophosphorylcholine (cadmium chloride complex), phosphorylcholine (calcium salt), betaine, and choline chloride were purchased from Sigma Chemical Co., St. Louis, Mo. Acetyl choline bromide was obtained from Eastman Kodak Ltd., Rochester, N.Y., and [Me-^{14}C]choline from Amersham/Searle Corp., Chicago, Ill. The salts of GPC and PC were converted to their free forms by being treated with a mixture of Amberlite IRC 50 and Amberlite IR-45 (ref. 12). Glycerophosphoryl[Me-^{14}C]choline was prepared by the method of alkaline deacylation of [Me-^{14}C]choline-labeled lecithin described by DAWSON[13]. Phosphoryl[Me-^{14}C]choline was prepared by the action of phospholipase C from *Clostridium welchii* on [Me-^{14}C]choline-labeled lecithin. Thin-layer chromatography (TLC) on 0.25-mm cellulose plates in the solvent system ethanol–0.88 ammonia–water (6:3:1, by vol.) (ref. 14) revealed that in both cases $>98\%$ of the applied radioactivity co-chromatographed with authentic samples of GPC or PC.

Radioactivity in column effluents was assayed in a Packard Tri-Carb liquid scintillation spectrometer after the addition of 10 ml of Aquasol (New England Nuclear, Boston, Mass.) per ml aqueous effluent. Choline and betaine were also assayed colorimetrically as their periodide derivatives by the method of SPEED AND RICHARDSON[15].

Results and discussion

The separation of GPC, PC, betaine, and choline was effected on columns (0.8 (I.D.) × 7 cm) of Dowex 50W. The samples (2 μmoles of each) were normally applied in a volume of 4–5 ml of water, and the column was washed successively with 25 ml of water, 90 ml of 0.4 *N* HCl, and 30 ml of 3 *N* HCl. As Fig. 1 illustrates, each of the four compounds was eluted from the column in separate peaks. Whereas GPC, PC, and betaine were completely resolved from each other, a small spillage of 3–4 % of the betaine into the choline fraction was observed. Generally no choline was

eluted in the first 90 ml of 0.4 *N* HCl. When, however, the column was washed with more than 90 ml of 0.4 *N* HCl, choline was eluted between 90–140 ml with a peak between 110–120 ml. In practice the column was washed with 90 ml of 0.4 *N* HCl, after which the acid concentration was increased to 3 *N*. Choline was completely eluted in 30 ml of the latter.

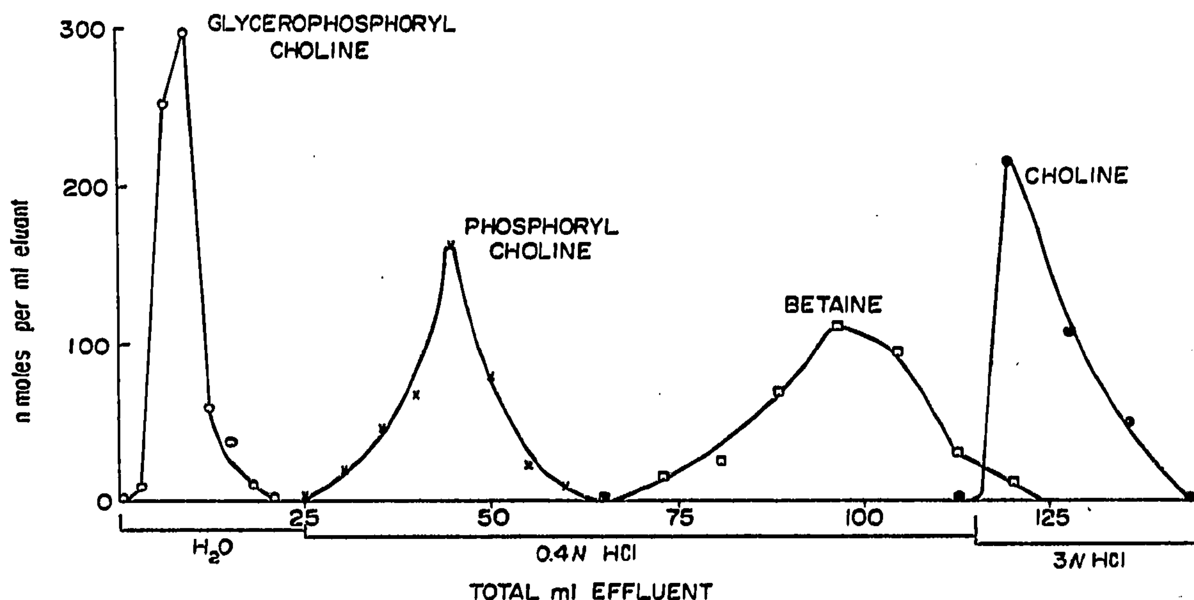

Fig. 1. Separation of glycerophosphorylcholine, phosphorylcholine, betaine, and choline on a column (0.8 (I.D.) × 7 cm) of Dowex 50W eluted with 25 ml of water, 90 ml of 0.4 *N* HCl, and 30 ml of 3 *N* HCl.

Similar separations were also achieved when mixtures containing 2 µmoles each of GPC, PC, betaine, and choline were applied to the columns or when 0.1 µmoles of radio-labeled GPC, PC, or choline was added to 2 µmoles of each of the other three compounds. Recoveries of material from the columns were > 98%.

Studies on the elution profile of acetyl choline, a known product of choline metabolism in neural tissue, demonstrated that this compound resembles choline in its affinity for the ion-exchange resin and is eluted in the 3 *N* HCl fraction. These two compounds were, however, easily separated from one another by cellulose-impregnated TLC in the solvent system 0.2 *M* ammonium acetate (pH 4.8)–acetone (20:80, by vol.) (ref. 16). Although the chromatographic properties of betaine aldehyde, an intermediate in the oxidation of choline to betaine, were not investigated, the data of SPEED AND RICHARDSON[15] indicate that it is probably also present in the 3 *N* HCl fraction.

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1 M. WAITE, G. L. SCHERPHOF, F. M. G. BOSHOUWERS AND L. L. M. VAN DEENEN, *J. Lipid Res.*, 10 (1969) 411.
2 S. EISENBERG, Y. STEIN AND O. STEIN, *J. Clin. Invest.*, 48 (1969) 2320.
3 H. HORTNAGL, H. WINKLER AND H. HORTNAGL, *Eur. J. Biochem.*, 10 (1969) 243.
4 D. R. ILLINGWORTH AND J. GLOVER, *Biochem. J.*, 115 (1969) 16P.
5 O. W. PORTMAN, *Adv. Lipid Res.*, 8 (1970) 41.
6 S. FOWLER AND C. DE DUVE, *J. Biol. Chem.*, 244 (1969) 471.
7 R. M. C. DAWSON, *Biochem. J.* 62 (1956) 689.
8 G. R. WEBSTER, E. A. MARPLES AND R. H. S. THOMPSON, *Biochem. J.*, 65 (1957) 374.
9 K. A. LLOYD-DAVIES, R. H. MICHELL AND R. COLEMAN, *Biochem. J.*, 127 (1972) 357.
10 E. R. WONG AND W. THOMPSON, *Biochim. Biophys. Acta*, 269 (1972) 259.
11 G. R. WEBSTER AND M. COOPER, *J. Neurochem.*, 15 (1968) 795.
12 E. BAER AND R. ROBINSON, *Can. J. Biochem.*, 45 (1967) 1747.
13 R. M. C. DAWSON, *Biochim. Biophys. Acta*, 14 (1954) 374.
14 R. M. C. DAWSON, A. T. MANN AND I. G. WHITE, *Biochem. J.*, 65 (1957) 627.
15 D. SPEED AND M. RICHARDSON, *J. Chromatogr.*, 35 (1968) 497.
16 I. DIAMOND AND E. P. KENNEDY, *J. Biol. Chem.*, 244 (1969) 3258.

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