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1 A. HOFMANN AND A. TSCHERTER, *Experientia*, 16 (1960) 414.
2 A. HOFMANN AND A. CERLETTI, *Deut. Med. Wochschr.*, 86 (1961) 885.
3 A. HOFMANN, *Planta Med.*, 9 (1961) 354.
4 W. A. TABER AND R. A. HEACOCK, *Can. J. Microbiol.*, 81 (1962) 137.
5 K. GENEST, *J. Chromatog.*, 19 (1965) 531.

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## DEAE Sephadex LH-20, a new chromatographic medium for the fractionation of acidic lipids

Brain phosphoinositides have been separated on DEAE cellulose with a salt gradient in a mixture of chloroform and methanol[1]. Because cellulose columns are difficult to pack with organic solvents, an exchange medium more compatible with lipid solvents was sought to be used for the application of this technique to the general fractionation of acidic lipids. DEAE Sephadex LH-20 has been prepared, and its use in the separation of the phosphoglycerides of *Escherichia coli* is described here. Conditions for the fractionation of more complex lipid mixtures with this exchanger are currently being investigated.

*Experimental*

Diethylaminoethoxypropylated Sephadex (DEAE Sephadex LH-20) was prepared from Sephadex LH-20 (Pharmacia Fine Chemicals) by the method described by PETERSON AND SOBER[2] for the preparation of DEAE cellulose. Titration of samples of the product in the $OH^-$form in methanol with HCl required 1.3 mequiv./g. The exchanger was stored in the formate form.

*E. coli* lipids were extracted as described elsewhere[3] and were separated into neutral and complex lipid fractions by chromatography on silicic acid[4]. All organic solvents and other chemicals were reagent grade and were used without further treatment.

Columns were packed with a slurry of DEAE Sephadex LH-20 in chloroform–methanol (1 : 1) which had been made 1.0 *M* with formic acid and 0.5 *M* with ammonium hydroxide by the addition of 90% formic acid and 30% ammonium hydroxide solutions, respectively. A 18 × 0.8 (I.D.) cm column was used for 2 g of exchange

medium. This column was fitted with Teflon 1.0–0.2 cm reducing union connectors (Beckman) at both ends and wads of glass wool were used to retain the medium. For the fractionations described here, elution of all solvents was done with a Phoenix Varigrad gradient pump (Phoenix Precision Instrument, Philadelphia). It should be noted that contrary to the manufacturer's specification, this pump, as supplied, is not suitable for use with solvents such as chloroform because of deterioration of an "O" ring in the check valve assembly. Satisfactory operation can be obtained if the check valve is removed and the pump is operated against a gravity pressure head of 1–2 ft. of solvent. The column was generally and most satisfactorily eluted from bottom to top.

The initial conversion of the medium to the formate form and subsequent regeneration of the column between runs was done by eluting the column in sequence with 50 ml of 0.5 *M* ammonium hydroxide–1.0 *M* formic acid in chloroform–methanol (1:1) and 50 ml of chloroform–methanol (1:1). Lipid samples were applied to the column in chloroform–methanol (1:1) with a Teflon sample injection valve (Chromatronix, Inc., Berkeley, Calif.). Simple lipids, if present, and neutral complex lipids were eluted with 50 ml of chloroform–methanol (1:1). The acidic complex lipids were eluted with an ammonium formate gradient in chloroform–methanol (1:1) run up to a concentration of 0.02 *M*. The gradient was formed by the differential pumping and mixing of chloroform–methanol (1:1) and the same solvent made to 0.02 *M* with ammonium formate. The latter solvent was prepared by adding equivalent amounts of formic acid and ammonium hydroxide to give the specified concentration. A total of 120 ml of solvent was used in the gradient. All solvents were pumped at a flow rate of 0.67 ml/min. A total of approximately 42 4-ml fractions were collected and 1-ml aliquots were assayed for phosphorus[5]. Appropriate fractions were combined for each compound and the homogeneity and identity of each was checked by analysis of the deacylation products[5] and by thin-layer chromatography[6].

*Results and discussion*

A typical fractionation of *E. coli* phosphoglycerides on DEAE Sephadex LH-20 is shown in Fig. 1. The three major fractions in the order of elution were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Recoveries were in the order of 100% of the phosphorus applied to the column in six separate runs with loadings from 5 to 40 μmoles lipid phosphorus per g exchange medium. The combined fractions for each peak of each of these six columns when analyzed by ion-exchange chromatography of deacylation products[5] were found to have only trace amounts of phospholipids other than the major component. All fractions appeared completely homogeneous when chromatographed on Silica Gel G thin-layer plates[6]. In three runs on *E. coli* phospholipid preparations that also contained phosphatidylglycerol phosphate (less than 0.5% of total lipid phosphorus) this compound was eluted in the same fractions as diphosphatidylglycerol. When total *E. coli* lipids were chromatographed, simple lipids (as detected on thin-layer chromatograms) were eluted from the column along with phosphatidylethanolamine. Otherwise no change in the elution pattern occurred.

Columns were regenerated *in situ* after each fractionation and 30 runs have been made on a single column with no change in performance. The solvent can be varied from 100% chloroform to 100% methanol without affecting the flow rate, however,

with high concentrations of chloroform the medium floats. In chloroform–methanol (1:1), concentrations of up to 2 *M* ammonium formate have been used without changing the flow rate. This is in marked contrast with DEAE Sephadex. In extensive experience with silicic acid eluted with combinations of chloroform and methanol with and without added water or ammonium hydroxide no comparable fractionation of *E. coli* phosphoglycerides was achieved.

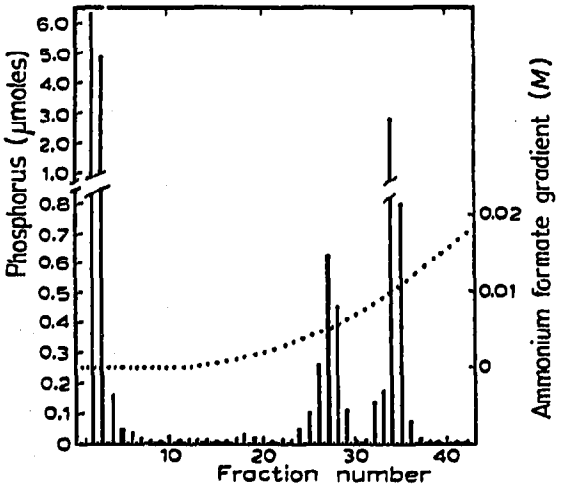


Fig. 1. Fractionation of *E. coli* phosphoglycerides on DEAE Sephadex LH-20 eluted with a gradient of ammonium formate in choroform–methanol (1:1). A 2-g, 18 × 0.8 cm (bed volume) column was used with a loading of 10 μmoles of lipid phosphorus per g. Solvent was pumped ascending with a flow rate of 0.67 ml/min and collected in 4-ml fractions.

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1 H. S. HENDRICKSON AND C. E. BALLOU, *J. Biol. Chem.*, 239 (1964) 1369.
2 E. A. PETERSON AND H. A. SOBER, *Biochem. Prepn.*, 8 (1961) 39.
3 C. L. RANDLE, P. W. ALBRO AND J. C. DITTMER, *Biochim. Biophys. Acta*, 187 (1969) 214.
4 B. BORGSTROM, *Acta Physiol. Scand.*, 25 (1952) 101.
5 J. C. DITTMER AND M. A. WELLS, *Methods Enzymol.*, 14 (1969) 482.
6 J. C. DITTMER AND R. L. LESTER, *J. Lipid Res.*, 5 (1964) 126.

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