peak about 20 % of the protein of the starting material appears together with about 80 % of the plasminogen. Consequently the starting material has been purified 4 times. This means an increase in purity of 16- to 20-fold as compared to Fraction III and the yield from Fraction III is about 50 %.

The reason for this behaviour of plasminogen is not understood. Attempts to adsorb the plasminogen to CM-cellulose equilibrated with buffers of pH 8.9 containing lysine have not been successful, which indicates that there is no reversal in the electric charge of the plasminogen.

1. Remmert, L. F. and Cohen, P. *J. Biol. Chem.* **181** (1949) 431.
2. Kline, D. L. *J. Biol. Chem.* **204** (1953) 949.
3. Müllertz, S. and Lassen, M. *Proc. Soc. Exptl. Biol. Med.* **82** (1953) 264.
4. Müllertz, S. *Biochem. J.* **61** (1955) 424.
5. Christensen, L. R. *J. Clin. Invest.* **28** (1949) 163.
6. Oncley, J. L., Melin, M., Richert, D. A., Cameron, J. W. and Gross, P. M. *J. Am. Chem. Soc.* **71** (1949) 541.
7. Sober, H. A. and Peterson, E. A. *J. Am. Chem. Soc.* **78** (1956) 751.
8. Alkjaersig, N., Fletcher, P. and Sherry, S. *J. Biol. Chem.* **234** (1959) 832.
9. Herriott, R. M. *Proc. Soc. Exptl. Biol. Med.* **46** (1941) 642.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem.* **193** (1951) 265.

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Separation of Glycerol Ethers by Gas-liquid Chromatography

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In the course of studies [1,2] on the metabolism of the 1-mono-ethers of glycerol with hexadecyl, octadecyl and 9-octadecenyl alcohol, *i.e.* chimyl, batyl and selachyl alcohol, a method for the separation of the individual glycerol ethers was needed. This separation has also earlier been made with adsorption chromatography on alumina [3], but any separation of batyl from selachyl alcohol was not obtained.

The separation of methyl esters of high molecular weight fatty acids by gas-liquid chromatography (GLC) has been reported [4-6]. Since GLC permits microgram mixtures of methyl esters of fatty acids to be resolved, analysis of mixtures of glycerol ethers has been investigated.

The present paper describes the complete separation of chimyl, batyl and selachyl alcohol by GLC after acylation of the two hydroxyl groups of the glycerol ethers.

Apparatus and procedure. The GLC experiments were carried out with an Argon Pye Chromatograph. The temperature of operation was 218°C and the inlet pressure of the gas 72 cm Hg. The rate of flow of the carrier gas (Argon) was 20 cm³/min. The detector voltage was set on 1 250 V. Each 4-foot column was packed with 5 g of acid-washed, alkali-treated Celite (mesh 100—140). As stationary phase a polar polyester LHC-1R-296 (obtained from Cambridge Inc., Mass. U.S.A.) was used in the ratio Celite: stationary phase 4:1. Acylation of the two hydroxyl groups of the glycerol ethers was carried out with acetic anhydride in the presence of pyridine after boiling on a water bath for 12 h. The esterified mixture of glycerol ethers was isolated and dissolved in a few microlitres of ethyl acetate, and the solution taken up in a micropipette and applied to the column.

The retention volumes of the different glycerol ethers were determined using a mixture of known compounds (Table 1). The retention volumes are expressed relative to synthetic batyl alcohol, which was used as internal standard. When determining the retention volumes of the pure compounds, special care was taken not to overcharge the column in order to obtain symmetrical peaks on the chromatogram. The polyester column had a retention time of batyl alcohol diacetin of 49 min.

Table 1. GLC characteristics on LHC-1R-296 as stationary phase of three glycerol ethers after acylation. Temperature 218°C. Argon flow rate 20 cm³/min.

	Apparent retention volume relative to batyl alcohol-diacetin = 1.00
Chimyl alcohol	0.61
Batyl alcohol	1.00
Selachyl alcohol	1.11

Before having a gas-liquid chromatography technique available a similar separation was made by reversed phase partition chromatography using 50 % aqueous *iso*propanol as moving phase and 20 % chloroform in heptane as stationary phase. The band containing chimyl and selachyl alcohol was then subjected to renewed chromatography after hydrogenation, when the selachyl alcohol originally present is obtained as batyl alcohol which is easily separated from the chimyl alcohol.

Material. Hexadecyl-α-glyceryl ether (chimyl alcohol) and octadecyl-α-glyceryl ether (batyl alcohol) was prepared according to Holmes *et al.* [7]

Specimens of purified glycerol ethers mainly containing selachyl alcohol were prepared from tiger shark *(Galeocerdo curvier)* liver fat [1,2,6]. The samples obtained were run on the gas-chromatograph before and after hydrogenation.

This method has proved to be useful for biological work with glycerol ethers and it may also be used for purification of such substances.

Using the column and conditions described acylated monoglycerides of different saturated and unsaturated fatty acids can also be separated.

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1. Bergström, S. and Blomstrand, R. *Acta Physiol. Scand.* **38** (1956) 166.
2. Blomstrand, R. and Ahrens, E. H., Jr. *Proc. Soc. Exptl. Biol. Med.* **100** (1959) 802.
3. Prelog, V., Ruzicka, L. and Steinman, F. *Helv. Chim. Acta* **27** (1944) 674.
4. James, A. T. and Martin, A. J. P. *Biochem. J.* **63** (1956) 144.
5. Stoffel, W., Insull, W. and Ahrens, E. J., Jr. *Proc. Soc. Exptl. Biol. Med.* **99** (1958) 238.
6. Orr, C. H. and Callen, J. E. *J. Am. Chem. Soc.* **80** (1958) 249.
7. Holmes, N. N., Corbet, R. E., Geiger, W. B., Kornblum, N. and Alexander, W. *J. Am. Chem. Soc.* **63** (1941) 2607.
8. Karnovsky, M. L. and Brumm, A. F. *J. Biol. Chem.* **216** (1955) 689.

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Chromatography on ECTEOLA * of Sulphate Containing Mucopolysaccharides and Nucleotides

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During a study on the enzymatic synthesis of heparin [1] it was found difficult to remove traces of ^{35}S-containing nucleotides from mucopolysaccharides prepared by the cetylpyridinium method [2,3]. We therefore tried chromatography on ECTEOLA * cellulose for the separation of these substances. ECTEOLA has previously been found especially valuable for the chromatography of nucleotides and polynucleotides [4].

Fig. 1 shows the chromatographic behaviour of five different polysaccharides during step-wise elution from ECTEOLA. For good separations it was found necessary to work at a quite acid pH. In the experiments of Fig. 1 the increase in chloride concentration was obtained by adding equimolar amounts of NaCl and HCl. Similar chromatograms were obtained when a constant concentration of 0.05 M HCl was used in all steps, while the chloride concentration was regulated by increasing the amounts of NaCl in the eluent. $CaCl_2$ could be used instead of NaCl. $CaCl_2$ can be removed from the polysaccharide fractions by extraction with alcohol-ether. It interferes, however, strongly with the carbazol reaction [5] and it was therefore not used extensively. The recovery of polysaccharides was found to be close to 100 %. All operations were performed at 0–5°C.

Hyaluronic acid, chondroitinsulfuric acid and heparin had very different affinities for ECTEOLA which allowed their separation.

* ECTEOLA cellulose (E. A. Peterson and H. A. Sober, *J. Am. Chem. Soc.* **78** (1956) 751) was obtained from Brown Company, Berlin, New Hampshire, USA.