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 [1,2,8]. 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.

This work is part of investigations supported by the *Swedish Medical Research Council.*

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.

Received August 12, 1959.

Chromatography on ECTEOLA * of Sulphate Containing Mucopolysaccharides and Nucleotides

NILS R. RINGERTZ and PETER REICHARD

Department of Cell Research and Genetics and Department of Chemistry I, Karolinska Institutet, Stockholm 60, Sweden

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.

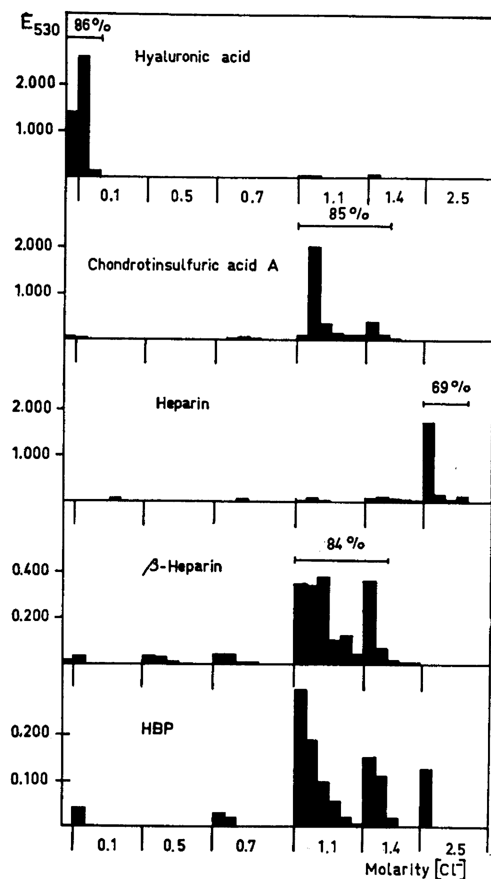


Fig. 1. Chromatography of mucopolysaccharides on ECTEOLA. The resin was washed with 5 liters of 0.5 M NaOH, 5 liters of 3 M NaCl and 3 liters of 0.1 M NaCl-HCl (1:1). Each polysaccharide (10—15 mg dissolved in 10—15 ml of 0.05 M NaCl-HCl) was adsorbed to a separate column (10 × 1 cm). The columns were eluted step-wise with buffers of increasing chloride concentrations (6 × 12 ml step). The fractions were analyzed by the carbazol method [5]. The figures over the main peaks in the chromatograms indicate the recovery of polysaccharides.

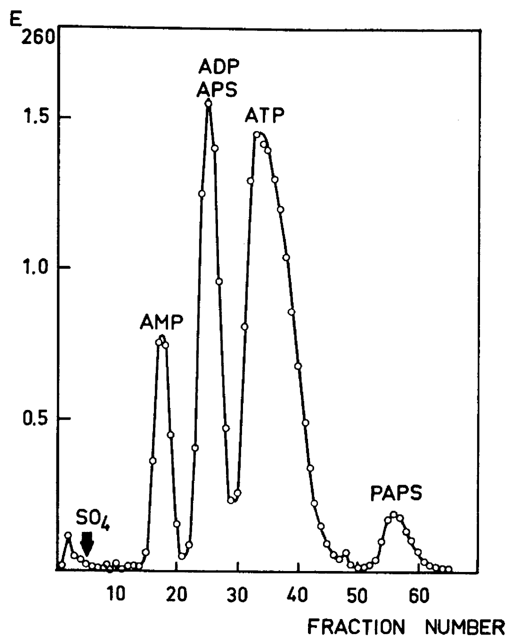


Fig. 2. Chromatography of nucleotides (20 μmoles, pH 7) on an ECTEOLA column (10 × 1 cm). Elution: Linear gradient; vessel 1 = 400 ml 0.01 M NaCl, 0.01 M tris-HCl, (tris-(hydroxymethyl)-aminomethane) pH 7; vessel 2 = 400 ml 0.4 M NaCl, 0.01 M tris-HCl, pH 7. Fractions 7 ml/30 min.

Such separations were actually performed with mixtures of these polysaccharides. It was also evident that the polysaccharides used were inhomogeneous. This was particulary true for a by-product obtained during heparin preparation. This polysaccharide (HBP) separated into four distinct fractions. Upon rechromatography the materials from each of the major fractions were recovered in their original positions. Table 1 shows some analytical data for these HBP-fractions. Evidently they differed largely in composition, *e.g.* as regards the relative proportions of glucosamine and galactosamine.

The anticoagulant activity [6] of heparin after chromatography using the most acid method (equimolar amounts of NaCl and HCl) was 94 Units/mg compared to 105 Units/mg before chromatography. Heparin recovered after elution according to the less acid method (0.05 M HCl) had an activity of 100 Units/mg. Because of the methodological errors involved, these three values are not considered to be significantly different. Analysis of heparin before and after chromatography showed that no loss of sulfate had occurred. This is further support for the absence of degradation.

Table 1. Analytical data for HBP polysaccharide and subfractions.

	SO_4[7]/aminosugar[8] molar ratio	glucosamine/ galactosamine[9] molar ratio	anticoagulant activity[6] units/mg
HBP polysaccharide	1.69	1.17	6
0.7 M subfraction	0.92	1.12	<4
1.1 M »	2.20	0.60	<2
1.4 M »	0.96	0.48	8
2.5 M »	1.39	4.83	12—20

Nucleotides were eluted at considerably lower salt concentrations. Adenosine 5'-phosphosulfate (APS) * and adenosine 3'-phosphate-5'-phosphosulfate (PAPS) * could be separated from adenosine 5'-phosphate and adenosine 5'-triphosphate at neutral pH. (Fig. 2). The recovery of nucleotides from the columns was close to 100 %. Chromatography at acid pH caused some degradation of labile nucleotides but served the purpose of removing nucleotides from mucopolysaccharides.

Acknowledgements. We thank Dr. S. Gardell, Dr. B. Högberg and Vitrum Company for generous gifts of mucopolysaccharides. The α-heparin was a gift from Dr. R. W. Jeanloz to Dr. S. Gardell. We also wish to thank prof. E. Jorpes for advice and help in the determination of anticoagulant activity.

1. Ringertz, N. R., Reichard, P. and Roden, L. *Unpublished.*
2. Scott, J. E. *Biochem. J.* **62** (1956) 31 P.
3. Scott, J. E. in Glick, D., Ed., *Methods of Biochemical Analysis*, Interscience Publishers Inc. New York. *To be published.*
4. Rosenkranz, H. S., Bendich, A., Pahl, H. B., Korngold, G. C. and Fresco, J. R. *J. Am. Chem. Soc.* **80** (1958) 3949.
5. Dische, Z. *J. Biol. Chem.* **167** (1947) 189.
6. Studer, A. and Winterstein, A. *Helv. Physiol. et Pharmacol. Acta* **9** (1951) 6.
7. Dodgson, K. S. and Spencer, B. *Biochem. J.* **55** (1953) 436.
8. Blix, G. *Acta Chem. Scand.* **2** (1948) 467.
9. Gardell, S. *Acta Chem. Scand.* **7** (1953) 207.

Received August 21, 1959.

* APS was synthesized by the carbodiimide method, Reichard, P. and Ringertz, N. R. *J. Am. Chem. Soc* **81** (1959) 878. PAPS was synthesized enzymatically from APS + ATP with a crude rat liver enzyme. Brunngraber, E. G. *J. Biol. Chem.* **233** (1958) 472.

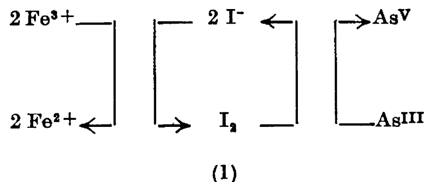
A Sensitive Colour Reaction for the Paper Chromatographic Detection of Iodide, Iodinated Tyrosines and Thyronines

ROLF GMELIN and ARTTURI I. VIRTANEN

Laboratory of the Foundation for Chemical Research, Biochemical Institute, Helsinki, Finland

The most sensitive chemical reaction for the detection of iodide, iodinated tyrosines and thyronines uses the catalytic action of traces of iodide upon the reduction of ceric sulphate by arsenic acid (Bowden *et al.*[1]). It allows the detection of 0.1 μg thyroxine, for instance. Modifications of the method have been proposed by Fletcher and Stanley [2], Dragunova and Langer [3], Gawienowski [4], and Mandl and Block [5].

We have observed that in the presence of catalytical amounts of iodide also the systems $Fe^{3+} \rightarrow Fe^{2+}$ and $Fe(CN)_6^{3-} \rightarrow Fe(CN)_6^{4-}$ can be brought in reaction with the system $As^{V} \rightarrow As^{III}$ (Formulas 1 and 2). The reaction becomes especially sensitive when ferri- and ferricyanide-ions are reacting simultaneously, catalyzed by iodide, upon arsenic acid, since the formed ferro- and ferrocyanide-ions, respectively are fixed immediately, forming the insoluble pigment, prussian blue (Formula 3).



(1)