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Estimation of radioactive acid mucopolysaccharides

For the isolation and fractionation of acid mucopolysaccharides the same methods employed for proteins can be utilized. For example, MEYER *et al.*[1] fractionated acid mucopolysaccharides by precipitating them from calcium acetate buffer by adding ethanol. SCOTT[2] showed that precipitates of different polyanions required different salt concentrations before they were dissolved and the critical salt concentrations for hyaluronate, chondroitin sulphates and heparin were found to be very different. ANTONOPOULOS *et al.*[3] investigated a fractionation procedure and developed a micromethod employing glass paper strips to fractionate mixtures containing 25–250 μg of each of the above-mentioned acid mucopolysaccharides.

Hyaluronate, chondroitin sulphate and heparin display different affinities for ECTEOLA-cellulose, thus allowing their separation by elution with salt solutions of varying concentrations at an acid pH[4]. SCHMIDT[5] used DEAE-Sephadex for the fractionation of acid mucopolysaccharides, and KERBY[6] used paper chromatography to separate heparin and chondroitin sulphate. CASTOR AND DORSTEWITZ[7] showed that it was also possible to separate different chondroitin sulphates by paper chromatography.

GARDELL *et al.*[8] separated chondroitin sulphate and hyaluronate by electrophoresis in Hyflo super-gel. Much smaller amounts of acid mucopolysaccharides can be separated by electrophoresis on cellulose acetate and the running time is much shorter than with paper electrophoresis[9,10]. The relative concentrations of different alcian blue-staining components of acid mucopolysaccharides were estimated by FRIMAN AND BRUNISH[11]. LEHTONEN[12] has characterized the separated fractions by gas chromatography.

Essentially all of the above-stated methods can be used for the fractionation and isolation of radioactive acid mucopolysaccharides as well. The literature shows that the incorporation of labelled sulphur into mucopolysaccharides is commonly used as a measure of mucopolysaccharide synthesis and the activity of the fibroblasts. Studies of the metabolism of mucopolysaccharides should generally involve methods for rapid isolation and estimation of small quantities of radioactive compounds. However, the preparation of samples for radioactivity measurements generally results in some loss of material. The purpose of this paper is to describe a method for determining the radioactivity of sulphated mucopolysaccharides directly from cellulose acetate sheets following electrophoresis, without time-consuming preparation of samples for radioactivity measurements.

0.1 ml of 0.9% NaCl solution, containing 20 μl [^{35}S]-sulphate (carrier-free, Radiochemical Centre, Amersham, England), was given intraperitoneally to rats which were sacrificed 6 h after the injection. The heads of long bones were collected and divided into fine pieces with scissors. The material was then homogenized in 0.01 *M* phosphate buffer, pH 6.8, for 60 sec with the Ultra-Turrax top drive homogenizer (Janke & Kunkel, KG., Stauffen i. Br., W. Germany). The hydrolysis of protein polysaccharides was performed with papain[2] (Verdauungskraft 1:350, E. Merck AG, Darmstadt, W. Germany), and the mucopolysaccharides were precipitated with ethanol, dissolved in distilled water, reprecipitated with 1% cetylpyridinium

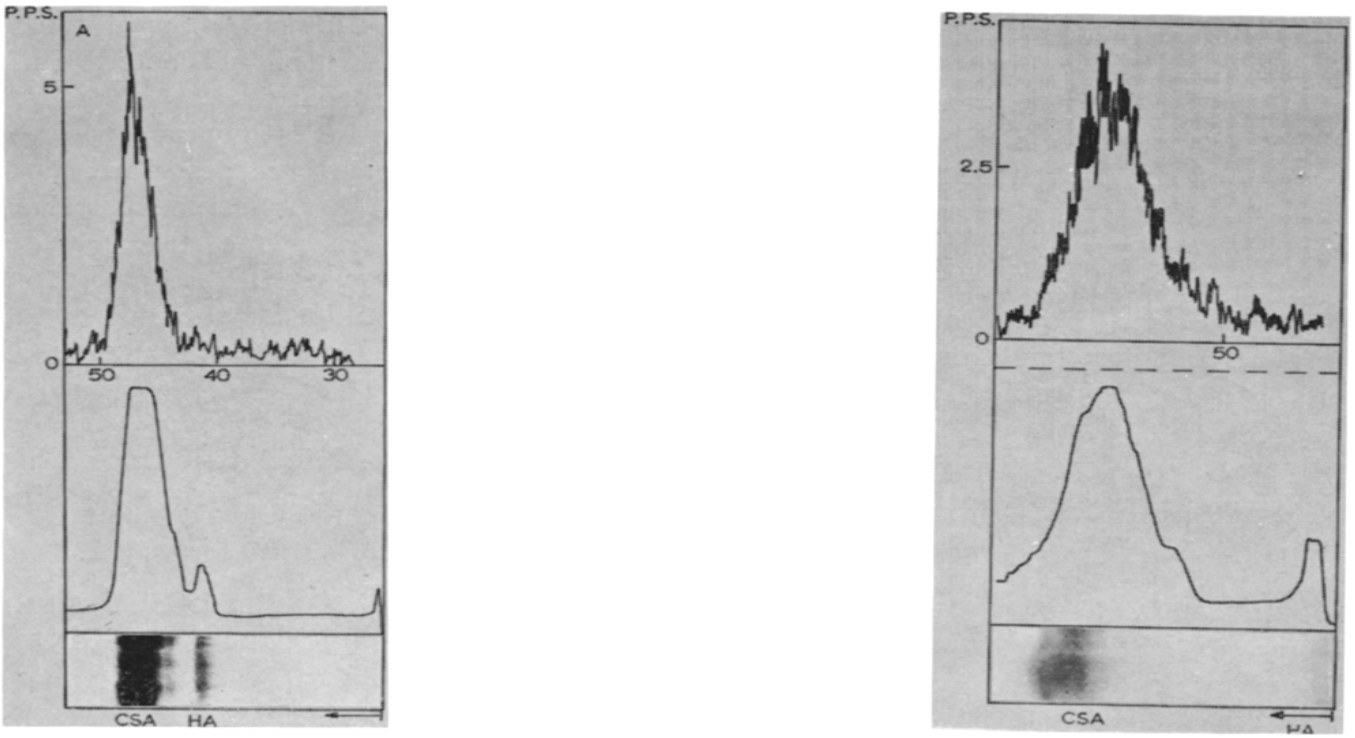

Fig. 1. Radioscans of acid mucopolysaccharides separated by electrophoresis on cellulose acetate sheets. The instrument settings used were: operation voltage, 3040 V; time constant, 10 sec; sensitivity 800 mV; scanning speed, 120 mm/h; slit width, 2 mm, without window; measuring range, 10 p.p.s. The Berthold ratemeter was replaced with an Ekco Ratemeter M 5190 (Ekco Electronics Ltd., Essex, England). (A) Separation of acid mucopolysaccharides in barbiturate buffer. Upper curve: determination of radioactivity; lower curve: densitogram of the Alcian Blue-stained fractions; the electropherogram: separation of acid mucopolysaccharides on acetate cellulose sheet. The fastest fraction had the same mobility as the chondroitin sulphates (CSA). The fraction with slower mobility represented hyaluronate (HA). (B) Separation of acid mucopolysaccharides in 0.5 *M* formic acid. In this buffer the sulphated mucopolysaccharides displayed a mobility different from that in barbiturate buffer. Other explanations are the same as for (A).

chloride (CPC) (Recip AB, Stockholm, Sweden)[2]. The CPC precipitate was dissolved in 2 *M* $MgCl_2$ solution, and the acid mucopolysaccharides were precipitated again with ethanol and dissolved finally in distilled water.

The electrophoretic fractionation of the acid mucopolysaccharides was carried out with cellulose acetate membranes (Oxoid®, Courtaulds Ltd., Coventry, England) in barbiturate buffer (pH 8.65, $\mu = 0.125$) and in 0.5 *M* formic acid (pH 3.0). The electrophoresis was carried out for 30 min at an operational voltage of 110 V. The acetate membranes were stained with a 1% solution of Alcian Blue (George T. Gurr Ltd., London, England) in 25% acetic acid[10]. The colour intensity of the stained mucopolysaccharide fractions was determined with a Chromoscan Double Beam Densitometer (Joyce & Loebl Co. Ltd., Gateshead, England). The distribution of radioactivity on the sheets was finally determined with a thin-layer scanner and a methane gas glow counter (Laboratorium Prof. Dr. Berthold, 7547, Wilbad, W. Germany) directly from the cellulose acetate strips. The results are given in Fig. 1, which shows that the fractions detected with Alcian Blue coincided with the radioactivity in the sheet, indicating the incorporation of labelled sulphate into acid mucopolysaccharides of the epiphyseal cartilage. The specific activity of the separated

acid mucopolysaccharides can be estimated by using labelled sulphur having a higher specific activity or simply by increasing the amount of labelled sulphur injected. This results in scanning curves without too much background disturbance.

This method has been used in our laboratory in the investigation of some aspects of the biosynthesis of chondroitin sulphates. The technique described here has some advantages because analytical values for the ratio of sulphate to hexosamine in many experiments do not correspond to the definition that one disaccharide residue of chondroitin sulphates contains one ester sulphate group[13]. In addition, in electrophoresis the mobility of chondroitin sulphates depends on the degree of sulphation[14], so that this method may be used when investigating the degree of sulphation of acid mucopolysaccharides.

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Institute of Dentistry,
University of Turku,
Turku 3 (Finland)

KEIJO U. PAUNIO
KAUKO K. MÄKINEN

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