## Solubility of Sodium Urate in the Presence of Chondroitin-4-sulphate

Recent work has shown that the presence of dextran decreases the solubility of macromolecules, such as proteins[1]. Since the relative decrease in solubility is a function of both protein size and polysaccharide concentration, the phenomenon can be interpreted as a steric exclusion of protein from a portion of the solvent within the domain of the polysaccharide chains. If this is the primary mechanism, then low-molecular-weight substances should be similarly effected provided that the polysaccharide concentration is sufficiently high.

It is well known that, in gout, crystals of sodium urate are deposited in connective tissues[2], some of which, as, for example, cartilage[3], have a very high polysaccharide content. For this reason, it was of special interest to use sodium urate and chondroitin-4-sulphate (CS) in an investigation to ascertain whether polysaccharides materially influence the solubility of low-molecular-weight substances.

The sodium salt of CS was isolated from bovine nasal cartilage according to the procedure developed by Scott[4] (nitrogen, 2·89 per cent; sulphur, 5·61 per cent; molecular weight, 1·5–2 × $10^4$). Solutions of CS varying in concentration from 6·3 to 18·8 g/100 ml. were prepared in a medium containing 0·1 M *p*H 7·6 sodium phosphate buffer and 25 per cent (v/v) saturated 5·7-dichloro-8-quinolinol, a bacteriostat and chelating agent. Aliquots of 4 ml. were added to test-tubes containing 15 mg uric acid. After having been shaken for 48 h at 37° C, the samples were centrifuged at the same temperature for 15 min at 20,000 r.p.m. The supernatants were diluted with water, and the concentration of urate was estimated spectrophotometrically from the extinction at 290 mμ using CS solutions of comparable concentrations as blanks. The relative solubility of urate in the presence of CS is shown in Fig. 1, where the solubility in the absence of CS has been taken as unity. As can be seen, the polysaccharide exerts a marked effect.

In order to test the exclusion hypothesis, an equilibrium dialysis was performed using the technique described by Ogston and Phelps[5]. CS and buffer solutions, identical with those used in the solubility investigations, were separated by membranes (dialysis tubing) impermeable to CS but permeable to sodium urate. Uric acid was added and allowed to equilibrate between the two solutions at 37° C. After equilibrium was attained the urate concentrations on both sides of the membrane were estimated by ultra-violet photometry and the CS concentrations were evaluated from dry weight determinations. The partition coefficient of urate between CS and buffer solutions can be regarded as a measure of the relative volume available for urate in the CS solution. The close agreement between the relative solubility of sodium urate in CS solutions and the partition coefficients calculated from dialysis experiments is readily apparent in Fig. 1, where both sets of data are plotted. Calculation shows that the volume excluded to sodium urate by CS is approximately 7 ml./g CS at low polysaccharide concentrations.

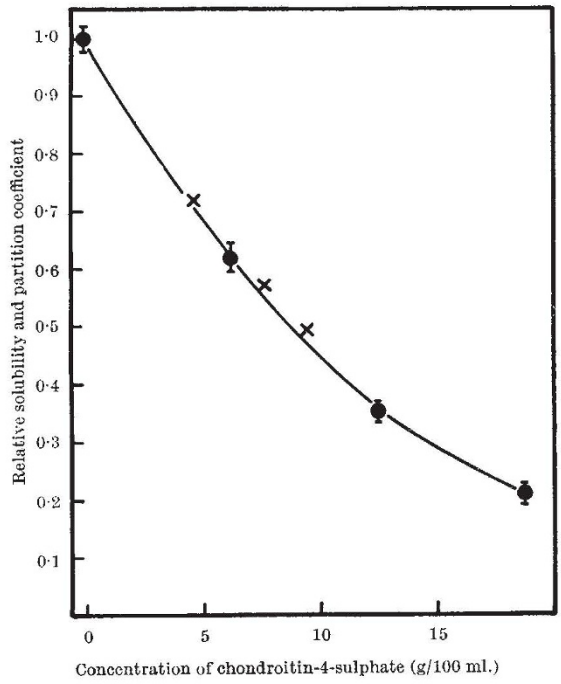


Fig. 1. Relative solubility of sodium urate in the presence of chondroitin-4-sulphate (●, each point represents six determinations) and the partition coefficient of urate between a chondroitin-4-sulphate solution and a buffer (×)

The mechanism proposed from these *in vitro* investigations could well account for the decreased solubility of urates in the connective tissue in gout, but only if the urate is produced within or in the neighbourhood of the tissue. For if urates are introduced into connective tissue via a diffusion equilibrium between the blood stream and the tissue, then one should expect to find a lower concentration of urate in connective tissue than in blood.

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## Acid Mucopolysaccharides of Human Aortic Adventitia

The presence of hyaluronic acid and chondroitin sulphates in the intima-media layers of human aortic tissue was first demonstrated in this department by chemical investigations conducted by Kirk *et al.*[1–3]. These observations have been confirmed in subsequent investigations by Kaplan and Meyer[4] and by Buddecke[5], and the isolation of heparitin sulphate from the human abdominal aorta has been reported by Linker *et al.*[6]. Investigations by Sundberg[7] have revealed the presence of an appreciable number of mast cells in the adventitia of the human abdominal aorta; but no chemical assays of the mucopolysaccharides in the aortic adventitia have as yet been reported.

This communication is a preliminary report of the determination of acid mucopolysaccharides in the adventitia of the human thoracic aorta by the use of the quaternary ammonium ion fractionation of these compounds. Eight samples of human adventitial tissue, derived from one or from several individuals, were included in the investigation.

The mucopolysaccharides were isolated from the tissue by the methods of Dyrbye and Kirk[8] and Schiller *et al.*[9]; the quantity of acid mucopolysaccharide material extracted from single or pooled adventitial samples averaged 1·6 milligrams per gram of wet tissue. The isolated mucopolysaccharides were afterwards precipitated as cetylpyridinium complexes and were fractionated by selective solubilities of sodium chloride by use of a modification of the method of Schiller *et al.*[9]; the sodium chloride concentrations were increased by steps in 0·2 M increments. This modification resulted in a mucopolysaccharide recovery close to 96 per cent. For comparative purposes, 2 mucopolysaccharide samples obtained from