IC-13 The composition of bone antler and periosteum
demineralised in organic solvents.

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Bone is easily demineralised in organic solvents using alkylammonium salts of EDTA (1). No hydroxyproline or hexosamine is extracted in the process and the matrix yields about 10 times the chondroitin sulphate compared with routine aqueous EDTA methods. 75% of the bone sialic acid is left in the matrix (Scott & Kyffin unpublished work).

Bovine shin bone was demineralised at 37°C in 80% ethanol-H₂O containing 0.5 M trimethylammonium E.D.T.A. The matrix was digested with papain at 65°C (2) and the polyanions were recovered with CPC (2). Fractionation with CPC in 0.2 M Na₂SO₄ yielded hyaluronate in the supernatant and sulphated polyanions in the precipitate. These were fractionated in EtOH-Ca acetate systems and the fractions identified by electrophoresis in Ba acetate buffer. Quantitation was by dissolution of the Alcian blue stained bands in dimethyl sulfoxide (3). The results for bone, antler and periosteum are shown in the figure.

Dermatan sulphate, heparan sulphate and hyaluronate were identified, for the first time in bone. Keratan sulphate was not found.

The matrix decalcified in organic solvents gives very 'crisp' histochemical pictures of the localisation of AGAG, using the Alcian blue-MgCl₂-CEC techniques (4). The PAS reaction of the ethanolic-EDTA treated bone is markedly stronger than that of the aqueous EDTA demineralised bone.

Fig. 1. Composition of demineralised matrices.

References